DISSERTATION ON "EVALUATION OF NEUROPROTECTIVE EFFECT OF CELASTRUS PANICULATUS ON COGNITION IMPAIRMENT CAUSED BY PHENYTOIN IN SWISS ALBINO MICE"

Dissertation submitted to

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY

In partial fulfillment of the regulations

For the award of the degree of

M.D. PHARMACOLOGY – BRANCH – VI

DHANALAKSHMI SRINIVASAN MEDICAL COLLEGE AND HOSPITAL, PERAMBALUR – 621212

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May – 2019

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This is to certify that the dissertation titled **"EVALUATION OF NEUROPROTECTIVE EFFECT OF CELASTRUS PANICULATUS ON COGNITION IMPAIRMENT CAUSED BY PHENYTOIN IN SWISS ALBINO MICE"** is a bonafide research work of

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Dear Dr. M. Venkatesh Perumal, P. Muthukrishnan,

This is with reference to your application seeking financial assistance from the ICMR for MD/MS/DM/MCh dissertation thesis entitled "**Evaluation of neuroprotective effect of Celastrus paniculatus on cognition impairment caused by phenytoin in Swiss albino mice**".

I am glad to inform you that Director General, ICMR, based on the recommendation of Expert Committee, has sanctioned a sum of **₹. 50, 000/- (Fifty thousand only)** to you for providing an electronic and hard copy of your dissertation thesis to the ICMR. Mandatory requirement to avail this opportunity is to provide us with an undertaking duly forwarded through the guide, to the undersigned, enabling us to release the grant.

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With best wishes,

Yours faithfully,

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This is certify that the project title "Evaluation of Neuroprotective effect of Celastrus Paniculatus on Cognition Impairement caused by Phenytoin In Swiss Albino Mice". Has been approved by the IAEC.

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PLAGIARISM CERTIFICATE

This is to certify that this dissertation work titled **"EVALUATION OF NEUROPROTECTIVE EFFECT OF CELASTRUS PANICULATUS ON COGNITION IMPAIRMENT CAUSED BY PHENYTOIN IN SWISS ALBINO MICE"** of the candidate **Dr. M. Venkatesh Perumal** with registration Number **201616552** for the award of **M.D** in the branch of **Pharmacology**. I personally verified from the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from Introduction to Conclusion pages and result shows **8%** Plagiarism in the dissertation.

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ABBREVIATIONS

1.	PHT	-	Phenytoin
2.	AED	-	Anti-epileptic drugs
3.	AChE	-	Acetyl Cholinesterase
4.	CDC	-	Centre for disease control and prevention
5.	DSM	-	Diagnostic and Statistical Manual of Mental disorders
6.	CNS	-	Central Nervous System
7.	OECD	-	Organisation for Economic Co-operation and Development
8.	GABA	-	Gamma Amino Butyric Acid
9.	NMDA	-	N- Methyl D-Aspartate
10.	HI	-	Hemidesmus indicus
11.	PTZ	-	Pentylenetetrazole
12.	PIM	-	Piracetam
13.	CP	-	Celastrus paniculatus
14.	USA	-	United States of America
15.	ADP	-	Adenosine diphosphate
16.	ATP	-	Adenosine triphosphate
17.	AMP	-	Adenosine monophosphate
18.	MES	-	Maximal Electroshock Seizures
19.	UK	-	United Kingdom
20.	ADHD	-	Attention Deficit Hyperactivity Disorder
21.	5-HT	-	5- Hydroxytryptamine (Serotonin)
22.	DTNB	-	Dithiobis(z- Nitrobenzoic Acid)

23.	OPT	-	Ophthaldialdehyde
24.	DSMCH	-	Dhanalakshmi Srinivasan Medical College & Hospital
25.	CS	-	Conditioned stimulus
26.	US	-	Unconditioned Stimulus
27.	CAR	-	Conditioned Avoidance Response
28.	ER	-	Escape Response
29.	ICES	-	Increasing Current Electroshock Seizures
30.	HLE	-	Hindlimb Extension
31.	HCl	-	Hydrochloric acid
32.	NaOH	-	Sodium Hydroxide
33.	Na2So3	-	Sodium Sulfite
34.	EDTA	-	Ethylene Diamine Tetra Acetic acid
35.	ANOVA	-	Analysis of Variance
36.	RAM	-	Radial Arm Maze
37.	PCA	-	Pole climbing Apparatus
38.	OD	-	Optical Density
39.	NT	-	Neurotransmitters
40.	IAEC	-	Institutional Animal Ethics Committee

CONTENTS

INTRODUCTION

"*We must realize that cognitive hygiene is as important subject as oral hygiene for healthy and happy existence*" –**Aditya Ajmera**.

Cognition refers to an individual's thoughts, knowledge, interpretation, understanding and ideas himself and his environment. [1]If the disturbances occur in these areas it leads to cognitive impairment. Loss of memory and cognitive function affects people worldwide; such loss may be the result of different progressive neurological disorders of the brain. It affects both men and women and is common in the elderly. [2]

Cognitive deficit is one of the major problems associated with epilepsy, both the underlying pathology and drug therapy can lead to disturbances in cognitive function. Nootropics agents may to some extent correct some of the observed cognitive deficits. Piracetam (PIM) is well known for its anti-myoclonus activity and specific anti-amnesic activity in many experimental models is used for enhancing cognition Phenytoin is one drug used commonly as anti-convulsant which affect learning and memory.[3]It might be worthwhile to assess the use of nootropic agents as add-on in antiepileptic therapy for possible protection against cognitive deficits. The antiepileptic treatment may last a lifetime in most patients. This implies that the nootropic agents may also need to be given for long periods of time and have to be chosen very carefully.

Phenytoin (PHT) is one of the low-cost drug and widely prescribed antiepileptic agent (AED) known to cause cognitive impairment. Many studies have investigated the effect of phenytoin on learning, memory and psychomotor functions. Administration of phenytoin has been shown to significantly impair learning and memory.

Acetylcholine has been a special target for investigations for almost two decades because its deficit, among other factors, has been held responsible for senile dementia and other degenerative cognitive disorders, including Alzheimer's disease. Major importance has been relied on acetylcholine, because of the declining number of Acetylcholine receptors with advancing age. Inhibitors of Acetylcholine-esterase (AChE), which terminates the action of acetylcholine, have been special targets for development. *Celastrus paniculatus* was in use from time immemorial to treat brain related disorders and to enhance learning and memory.

The present study infer the following objectives which were derived from the paradigm explained above with a thrust on the understanding of the effect of *Celastrus paniculatus* seed oil (Jyothismati oil), a potential nervine on the central nervous system based on behavioral and biochemical study.

According to the ***Centre for Disease Control and prevention (CDC),*** Cognitive decline is defined as trouble remembering, learning new things, concentrating or making decisions that affect everyday life. Cognitive impairment ranges from mild to severe. As the condition develops, a person may notice changes in their cognitive function, but still have success accomplishing everyday activities and living independently. More severe types of impairment can impact a person's ability to control bodily movements,

understand the meaning or importance of something, as well as affect speech and writing abilities.[4]

Cognition includes attention, memory, language, orientation, praxis, executive function, judgment and problem solving. Disorders of cognitionare rarely restricted to impairments in only one or more above domains. Instead, these disorders tend to defy Occam's razor, challenging clinicians and nosologists with multiplicity, comorbidity and unclear boundaries. These concerns are most true in the elderly, the demographic group most at risk for cognitive disorders. Dementia in late life is particularly problematic in this regard. Existing, although often unrecognized, dementia is a major risk factor for superimposed delirium.

Certain dementias, such as dementia with lewy bodies or late stages of Alzheimer's disease, may have chronic clinical presentations virtually indistinguishable from delirium except for temporal onset and the lack of an identifiable acute source. Similarly, the course of nearly all subjects developing a progressive dementia is complicated by the onset of one or more distinct behavioral syndromes, including anxiety, depression, sleep problems, psychosis, and aggression. These symptoms can be as distressing and disabling as the primary cognitive disorder. Some of these behavioral syndromes, such as psychosis, may themselves result from independent underlying biology and may be additive with the primary neurodegenerative process.[5]

The boundaries between types of dementia and between dementia and normal aging can be similarly diffuse. The most common neuropathological presentations associated with dementia reveal mixtures of Alzheimer's disease, vascular and lewy body pathologies. Pure syndromes are relatively less common, although often the dementia is ascribed to one of the coexisting pathologies. Strategies regarding how to understand or reconcile multiple pathologies in the clinic are needed, although they lag behind. The development of such strategies would clearly be enhanced by a nosology within the coming version of Diagnostic and Statistical Manual of Mental Disorders (DSM) that does not preclude giving each specific diagnosis, although other central nervous system (CNS) or Axis I conditions are present.

The boundary between dementia and normal aging has also been recently blurred. The majority of community dwelling elders who have been carefully and clinically evaluated and found to lack signs of dementia will nevertheless have neuropathology lesions of Alzheimer's disease, infarction, or Lewy bodies at autopsy. Similarly, the advent of in vivo imaging of amyloid plaques indicates that many normal elderly and elderly with mild cognitive impairment may already have amyloid deposition throughout their cortex to an extent that is equivalent to that of many individuals with Alzheimer's disease has been defined by the presence of cognitive symptoms. Findings such as those above suggest that the time is coming when the situation will more resemble that for coronary artery disease, which is defined by the presence of plaques that may be silent or symptomatic. [5]

The demographic imperative with regard to cognitive disorders is clear. The population is rapidly aging and the age is the single largest factor for developing dementia and other cognitive and mental disorders. When mild cognitive impairment presents as an isolated amnestic symptom in late life, it strongly predicts subsequent onset of Alzheimer's disease, although other conditions such as focal brain lesions, metabolic disturbances and alcohol use may lead to isolated amnestic disorders. In practice mild cognitive impairment due to underlying Alzheimer's disease is more prevalent.

CAUSES OF COGNITIVE IMPAIRMENT:

- **Disease affecting cognitive impairment**:
  - **Neurodegenerative disorders**
  - **Vascular causes,**
  - **Neurological Disease**
  - **Nutritional Disorders**
  - **Endocrine**
  - **Infectious**
  - **Metabolic**
  - **Drugs &Toxins [6]**

PREVALENCE OF COGNITIVE IMPAIRMENT:

Cognitive impairment and dementia are increasing globally and predicted to increase proportionately more in developing regions. It is estimated that 35.6 million people are currently living with dementia worldwide and that the number will nearly double every 20 years, reaching 115.4 million in 2050, with the majority living in developing countries.[7] It has been estimated that in India, the population of those aged over 60 years will have increased from its level of 7.7% in 2001 to 12.30% by 2025 and there will be nearly 150 million elderly individuals. Cognitive disability or dementia is a relatively common disorder among the elderly. Most people with cognitive disability live in low- or middle-income countries (60% in 2001, estimated to rise to 71% by 2040); the rate of increase in cognitive disability over the decades is around 300% for India, whereas it is estimated to be only 100% in high-income countries.[7]

A neurologically degenerative disorder is the underlying cause in the majority of cases of significant cognitive decline.[8]*Dementia* represents a substantial financial burden on society, one that is similar to the financial burden of heart disease and cancer. In the aging population, cognitive functions represent a fundamental target that is receiving the attention of health systems. In 2009, the prevalence of dementia among the population aged 60 years and older ranged from 6.5% in France (and 6.4% in Italy) to 3.4% in India with an average of 5.5% for members of the Convention on the Organization for Economic Co-operation and Development (OECD).

In an analysis, 235 selected studies involved 44,854 patients with Dementia (mainly vascular dementia, Alzheimer disease and mild cognitive impairment) the

efficacy of symptomatic treatment for vascular dementia with Piracetam, Nimodipine, Aniracetam, Flunarizine, Vinpocetine, Hyperbaric oxygen, Oxiracetam and treatment with other alternative therapies including Acupuncture, Premarin, Statin, Butylphthalide, Donepezil, Huperzine A, and Lithium treatment were higher than those of other existing treatments for cognitive dysfunction[9]

Epilepsy is defined as "occasional sudden excessive, rapid and local discharges of grey matter resulting in intermittent and stereotyped disturbance in consciousness, behavior, emotion, motor function or sensation that on clinical grounds is believed to result from cortical neuronal discharge.[10]It is a neuropsychological disorder affecting millions of people in world level. Disturbance of naturally existing balance between the concentrations of inhibitory and excitatory neurotransmitters in central nervous system are assumed to be main cause of convulsive episodes. There are number of drugs available for the treatment of epilepsy in modern therapy. But the major disadvantages faced is their side effects and chronic toxicity.[11]

Approximately 50 million people currently live with epilepsy worldwide. The estimated proportion of the general population with active epilepsy (i.e. continuing seizures or with the need for treatment) at a given time is between 4 and 10 per 1000 people. The overall incidence is high in the first year, drops to a minimum in the third and fourth decades of life, and then increases again in later life. More than 75 percent of patients have their first seizure before 18 years of age and 12 to 20 percent have a familial incidence of seizures. Among adults the most common seizures are the complex

partial and generalized tonic clonic seizures. However, some studies in low- and middle-income countries suggest that the proportion is much higher, between 7 and 14 per 1000 people. Globally, an estimated 2.4 million people are diagnosed with epilepsy each year. In high-income countries, annual new cases are between 30 and 50 per 100 000 people in the general population. Hence these people who are on anticonvulsants were found to have cognition impairment as a sequelae.[12]

Epileptic seizures can be primary or secondary to a neurologic condition or reactive to a situational factor, such as sleep deprivation or drug withdrawal. It is the recurrent tendency to seize and status epilepticus is prolonged or repetitive seizures without intervening recovery. In this condition abnormal electrical discharges are due to hyperexcitable neurons with postsynaptic depolarization include changes in ionic conductance, decreased Gamma-Aminobutyric Acid (GABA) inhibition of cortical excitability and increased glutamate – mediated cortical excitation. In animals, alumina-induced membrane changes alter the ratio of intracellular to extracellular ionic concentration s and results in abnormal neuronal firing.

Kainic acid, a glutamate agonist, induces seizure through increased synaptic action at its N-mehtyl –D- aspartate (NMDA) receptors.[13]Much work is underway on potential antiepileptic drugs that may act through inhibition of this excitatory receptor mechanism. Hence finding out of anti-epileptic drugs becomes the essential target that too with least adverse effect profile and this leads for the accountability of this study by using the herbal drugs to obtain enhancement of cognition and anti-seizure activity.

HERBAL DRUGS AND COGNITION:

Herbalism (also **herbal medicine** or **phytotherapy**) is the study of botany and use of plants intended for medicinal purposes or for supplementing a diet. Plants have been the basis for medical treatments through much of human history and such traditional medicine is still widely practiced today. Modern medicine makes use of many plant-derived compounds as the basis for evidence-based pharmaceutical drugs.
Although phytotherapy may apply modern standards of effectiveness testing to herbs and medicines derived from natural sources, few high-quality clinical trials and standards for purity or dosage exist.

The use of **plants** for treatment in ailments in India dates back to prehistoric times. Ayurveda, an ancient traditional system of medicine that has been practiced in India since 200 B.C., employs a large number of medicinal plants used in prevention and treatment of wide number of diseases. One of these includes the plant *C. paniculatus*, known for the centuries as "Elixir of life". It is considered in Ayurveda to stimulate 'medha' (intellect) and promotes 'smruti' (memory) and so Ayurveda recognizes it as 'Jyotishmati'dose regimen. *C. paniculatus* may be employed as stimulant nerve tonic, rejuvenant, sedative, tranquilizer and diuretic. It is also used in the treatment of rheumatism, gout, leprosy, leucoderma, paralysis and asthma. Jyotishmati has been mentioned by Sushruta, Charaka and Vagbhatta as a remedy for mental illness. Charaka gave the decoction of the root or seed internally in prescriptions, as a brain tonic for headache, depression, swooning; as a laxative for cleaning digestive system. Sushruta prescribed seed oil internally for

neurological disorders, urinary infections, skin infections, intestinal parasites and externally for wound healing.

Leaves were used internally as a purgative. Chakradatta recommended fried leaves of Jyotishmati for inducing menstruation. The juice of leaves was also given in opium poisoning as a de-addiction aid.[14]

According to Ayurveda, depending upon the Medicinal Plants with neuro-protection include: Alzheimer's disease is an imbalance of *vata*, *pitta* and *kapha.* Medhya (intellectual promoting) herbs such as *Convolvulus microphyllus (C. pluricaulis), Centella asiatica, Bacopa monnieri, Acorus calamus* and *Celastrus paniculatus* are beneficial in cognitive disorders. Hemidesmus indicus(HI), commonly known as Indian sarsaparilla or Anantmool is a slender, laticiferous and twining shrub available over costal districts of Orissa and some greater part of India . Bacopa monniera is a reputed nootropic plant mentioned in Ayurveda for various disorder of Central nervous system.[15]

In our study we used Celastrus paniculatus oil which is well known for its cognitive benefits is taken for evaluation and a standard drug Piracetam, also an established drug in improving cognition impairment is also been evaluated. For this ailment a new drug molecule is inevitable, with this perspective this study was carried out.

SOME PLANTS USED AS MEMORY ENHANCERS:

Plants	Useful parts	Active constituents
Allium sativum	Bulb	Sallylcysteine
Bocopa monniera	Whole plant	Bacosides A & B
Celastrus paniculatus	Seeds	Celapagine & Celapanigine
Nicotiana tobaccum	Leaves	Nicotine
Withania somnifera	Roots	Withanolides
Ricinus communis	Beans	Ricinine
Salvia officinalis	Leaves	Monoferpenoid
Ginkgo biloba	Leaves	Ginkgolides
Huperzia serrate	Moss	Huperzine
Uncariato mentosa	Bulbs	Total alkaloids
Physostigma vennosam	Beans	Physostigmine
Acorus calamus	Rhizomes	Asarone & methyl isoeugenol
Terminalia chebula	Rhizome	Chebulic acid

[16]

AIM & OBJECTIVES:

1. To evaluate the cognition enhancement property of *Celastrus paniculatus* in phenytoin induced cognition impairment.

2. To determine the antiepileptic activity of *Celastrus paniculatus.*

3. To assess the hepatorenal toxicity of *Celastrus paniculatus*.

INTRODUCTION

"*We must realize that cognitive hygiene is as important subject as oral hygiene for healthy and happy existence*" –**Aditya Ajmera**.

Cognition refers to an individual's thoughts, knowledge, interpretation, understanding and ideas himself and his environment. [1]If the disturbances occur in these areas it leads to cognitive impairment. Loss of memory and cognitive function affects people worldwide; such loss may be the result of different progressive neurological disorders of the brain. It affects both men and women and is common in the elderly. [2]

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Certain dementias, such as dementia with lewy bodies or late stages of Alzheimer's disease, may have chronic clinical presentations virtually indistinguishable from delirium except for temporal onset and the lack of an identifiable acute source. Similarly, the course of nearly all subjects developing a progressive dementia is complicated by the onset of one or more distinct behavioral syndromes, including anxiety, depression, sleep problems, psychosis, and aggression. These symptoms can be as distressing and disabling as the primary cognitive disorder. Some of these behavioral syndromes, such as psychosis, may themselves result from independent underlying biology and may be additive with the primary neurodegenerative process.[5]

The boundaries between types of dementia and between dementia and normal aging can be similarly diffuse. The most common neuropathological presentations associated with dementia reveal mixtures of Alzheimer's disease, vascular and lewy body pathologies. Pure syndromes are relatively less common, although often the dementia is ascribed to one of the coexisting pathologies. Strategies regarding how to understand or reconcile multiple pathologies in the clinic are needed, although they lag behind. The development of such strategies would clearly be enhanced by a nosology within the coming version of Diagnostic and Statistical Manual of Mental Disorders (DSM) that does not preclude giving each specific diagnosis, although other central nervous system (CNS) or Axis I conditions are present.

The boundary between dementia and normal aging has also been recently blurred. The majority of community dwelling elders who have been carefully and clinically evaluated and found to lack signs of dementia will nevertheless have neuropathology lesions of Alzheimer's disease, infarction, or Lewy bodies at autopsy. Similarly, the advent of in vivo imaging of amyloid plaques indicates that many normal elderly and elderly with mild cognitive impairment may already have amyloid deposition throughout their cortex to an extent that is equivalent to that of many individuals with Alzheimer's disease has been defined by the presence of cognitive symptoms. Findings such as those above suggest that the time is coming when the situation will more resemble that for coronary artery disease, which is defined by the presence of plaques that may be silent or symptomatic. [5]

The demographic imperative with regard to cognitive disorders is clear. The population is rapidly aging and the age is the single largest factor for developing dementia and other cognitive and mental disorders. When mild cognitive impairment presents as an isolated amnestic symptom in late life, it strongly predicts subsequent onset of Alzheimer's disease, although other conditions such as focal brain lesions, metabolic disturbances and alcohol use may lead to isolated amnestic disorders. In practice mild cognitive impairment due to underlying Alzheimer's disease is more prevalent.

CAUSES OF COGNITIVE IMPAIRMENT:

- **Disease affecting cognitive impairment**:
  - **Neurodegenerative disorders**
  - **Vascular causes,**
  - **Neurological Disease**
  - **Nutritional Disorders**
  - **Endocrine**
  - **Infectious**
  - **Metabolic**
  - **Drugs &Toxins** [6]

PREVALENCE OF COGNITIVE IMPAIRMENT:

Cognitive impairment and dementia are increasing globally and predicted to increase proportionately more in developing regions. It is estimated that 35.6 million people are currently living with dementia worldwide and that the number will nearly double every 20 years, reaching 115.4 million in 2050, with the majority living in developing countries.[7] It has been estimated that in India, the population of those aged over 60 years will have increased from its level of 7.7% in 2001 to 12.30% by 2025 and there will be nearly 150 million elderly individuals. Cognitive disability or dementia is a relatively common disorder among the elderly. Most people with cognitive disability live in low- or middle-income countries (60% in 2001, estimated to rise to 71% by 2040); the rate of increase in cognitive disability over the decades is around 300% for India, whereas it is estimated to be only 100% in high-income countries.[7]

A neurologically degenerative disorder is the underlying cause in the majority of cases of significant cognitive decline.[8]*Dementia* represents a substantial financial burden on society, one that is similar to the financial burden of heart disease and cancer. In the aging population, cognitive functions represent a fundamental target that is receiving the attention of health systems. In 2009, the prevalence of dementia among the population aged 60 years and older ranged from 6.5% in France (and 6.4% in Italy) to 3.4% in India with an average of 5.5% for members of the Convention on the Organization for Economic Co-operation and Development (OECD).

In an analysis, 235 selected studies involved 44,854 patients with Dementia (mainly vascular dementia, Alzheimer disease and mild cognitive impairment) the

efficacy of symptomatic treatment for vascular dementia with Piracetam, Nimodipine, Aniracetam, Flunarizine, Vinpocetine, Hyperbaric oxygen, Oxiracetam and treatment with other alternative therapies including Acupuncture, Premarin, Statin, Butylphthalide, Donepezil, Huperzine A, and Lithium treatment were higher than those of other existing treatments for cognitive dysfunction[9]

Epilepsy is defined as "occasional sudden excessive, rapid and local discharges of grey matter resulting in intermittent and stereotyped disturbance in consciousness, behavior, emotion, motor function or sensation that on clinical grounds is believed to result from cortical neuronal discharge.[10]It is a neuropsychological disorder affecting millions of people in world level. Disturbance of naturally existing balance between the concentrations of inhibitory and excitatory neurotransmitters in central nervous system are assumed to be main cause of convulsive episodes. There are number of drugs available for the treatment of epilepsy in modern therapy. But the major disadvantages faced is their side effects and chronic toxicity.[11]

Approximately 50 million people currently live with epilepsy worldwide. The estimated proportion of the general population with active epilepsy (i.e. continuing seizures or with the need for treatment) at a given time is between 4 and 10 per 1000 people. The overall incidence is high in the first year, drops to a minimum in the third and fourth decades of life, and then increases again in later life. More than 75 percent of patients have their first seizure before 18 years of age and 12 to 20 percent have a familial incidence of seizures. Among adults the most common seizures are the complex

partial and generalized tonic clonic seizures. However, some studies in low- and middle-income countries suggest that the proportion is much higher, between 7 and 14 per 1000 people. Globally, an estimated 2.4 million people are diagnosed with epilepsy each year. In high-income countries, annual new cases are between 30 and 50 per 100 000 people in the general population. Hence these people who are on anticonvulsants were found to have cognition impairment as a sequelae.[12]

Epileptic seizures can be primary or secondary to a neurologic condition or reactive to a situational factor, such as sleep deprivation or drug withdrawal. It is the recurrent tendency to seize and status epilepticus is prolonged or repetitive seizures without intervening recovery. In this condition abnormal electrical discharges are due to hyperexcitable neurons with postsynaptic depolarization include changes in ionic conductance, decreased Gamma-Aminobutyric Acid (GABA) inhibition of cortical excitability and increased glutamate – mediated cortical excitation. In animals, alumina-induced membrane changes alter the ratio of intracellular to extracellular ionic concentration s and results in abnormal neuronal firing.

Kainic acid, a glutamate agonist, induces seizure through increased synaptic action at its N-mehtyl –D- aspartate (NMDA) receptors.[13]Much work is underway on potential antiepileptic drugs that may act through inhibition of this excitatory receptor mechanism. Hence finding out of anti-epileptic drugs becomes the essential target that too with least adverse effect profile and this leads for the accountability of this study by using the herbal drugs to obtain enhancement of cognition and anti-seizure activity.

HERBAL DRUGS AND COGNITION:

Herbalism (also **herbal medicine** or **phytotherapy**) is the study of botany and use of plants intended for medicinal purposes or for supplementing a diet. Plants have been the basis for medical treatments through much of human history and such traditional medicine is still widely practiced today. Modern medicine makes use of many plant-derived compounds as the basis for evidence-based pharmaceutical drugs.
Although phytotherapy may apply modern standards of effectiveness testing to herbs and medicines derived from natural sources, few high-quality clinical trials and standards for purity or dosage exist.

The use of **plants** for treatment in ailments in India dates back to prehistoric times. Ayurveda, an ancient traditional system of medicine that has been practiced in India since 200 B.C., employs a large number of medicinal plants used in prevention and treatment of wide number of diseases. One of these includes the plant *C. paniculatus*, known for the centuries as "Elixir of life". It is considered in Ayurveda to stimulate 'medha' (intellect) and promotes 'smruti' (memory) and so Ayurveda recognizes it as 'Jyotishmati'dose regimen. *C. paniculatus* may be employed as stimulant nerve tonic, rejuvenant, sedative, tranquilizer and diuretic. It is also used in the treatment of rheumatism, gout, leprosy, leucoderma, paralysis and asthma. Jyotishmati has been mentioned by Sushruta, Charaka and Vagbhatta as a remedy for mental illness. Charaka gave the decoction of the root or seed internally in prescriptions, as a brain tonic for headache, depression, swooning; as a laxative for cleaning digestive system. Sushruta prescribed seed oil internally for

neurological disorders, urinary infections, skin infections, intestinal parasites and externally for wound healing.

Leaves were used internally as a purgative. Chakradatta recommended fried leaves of Jyotishmati for inducing menstruation. The juice of leaves was also given in opium poisoning as a de-addiction aid.[14]

According to Ayurveda, depending upon the Medicinal Plants with neuro-protection include: Alzheimer's disease is an imbalance of *vata*, *pitta* and *kapha.* Medhya (intellectual promoting) herbs such as *Convolvulus microphyllus (C. pluricaulis), Centella asiatica, Bacopa monnieri, Acorus calamus* and *Celastrus paniculatus* are beneficial in cognitive disorders. Hemidesmus indicus(HI), commonly known as Indian sarsaparilla or Anantmool is a slender, laticiferous and twining shrub available over costal districts of Orissa and some greater part of India . Bacopa monniera is a reputed nootropic plant mentioned in Ayurveda for various disorder of Central nervous system.[15]

In our study we used Celastrus paniculatus oil which is well known for its cognitive benefits is taken for evaluation and a standard drug Piracetam, also an established drug in improving cognition impairment is also been evaluated. For this ailment a new drug molecule is inevitable, with this perspective this study was carried out.

SOME PLANTS USED AS MEMORY ENHANCERS:

Plants	Useful parts	Active constituents
Allium sativum	Bulb	Sallylcysteine
Bocopa monniera	Whole plant	Bacosides A & B
Celastrus paniculatus	Seeds	Celapagine & Celapanigine
Nicotiana tobaccum	Leaves	Nicotine
Withania somnifera	Roots	Withanolides
Ricinus communis	Beans	Ricinine
Salvia officinalis	Leaves	Monoferpenoid
Ginkgo biloba	Leaves	Ginkgolides
Huperzia serrate	Moss	Huperzine
Uncaria tomentosa	Bulbs	Total alkaloids
Physostigma vennosam	Beans	Physostigmine
Acorus calamus	Rhizomes	Asarone & methyl isoeugenol
Terminalia chebula	Rhizome	Chebulic acid

[16]

AIM & OBJECTIVES:

1. To evaluate the cognition enhancement property of *Celastrus paniculatus* in phenytoin induced cognition impairment.

2. To determine the antiepileptic activity of *Celastrus paniculatus.*

3. To assess the hepatorenal toxicity of *Celastrus paniculatus*.

REVIEW OF LITERATURE

Phenytoin was first prepared in 1908 by the German chemist Heinrich Biltz and found useful for epilepsy in 1936. It is one of the drugs in the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. It is not a Central nervous system depressant, some sedation occurs at therapeutic doses but does not increase with further dose, and rather toxic doses produce excitement and muscular rigidity. The most outstanding action is abolition of tonic phase of maximal electroshock seizures, with no effect on or prolongation of clonic phase. It limits the spread of seizure activity. Threshold for Pentylenetetrazole convulsions is not raised and tonic clonic epilepsy is suppressed.

Phenytoin (PHT) is oldest non-sedative anti-seizure drug, introduced in 1938 after a systematic evaluation of compounds such as Phenobarbital that altered electrically induced seizures in laboratory animals. It was known for decades as diphenyl hydantoin.[17] Many studies have interrogated the side effects of phenytoin. The treatment includes anticonvulsants such as phenytoin was found to have major adverse effects on memory, learning, and psychomotor functions, where by phenytoin, in both acute and chronic administration, has been associated with impairment in memory and learning.[18] Many epileptic patients suffer from cognitive impairment; both the underlying pathology and antiepileptic drug therapy can cause such deficits.

Phenytoin is one of the low-cost and widely prescribed antiepileptic drugs (AED) known to cause cognitive impairment. Many studies have investigated the effect of phenytoin on learning, memory and psychomotor functions. For an optimum antiepileptic therapy, it is desirable to have complete seizure control without interfering cognitive effects. A combination of antiepileptic drugs with known nootropic agents appears to be a promising research area for desirable seizure control with minimal/no memory deficit. A better approach would be to use an agent that not only corrects the cognitive disturbances but also provides seizure protection.

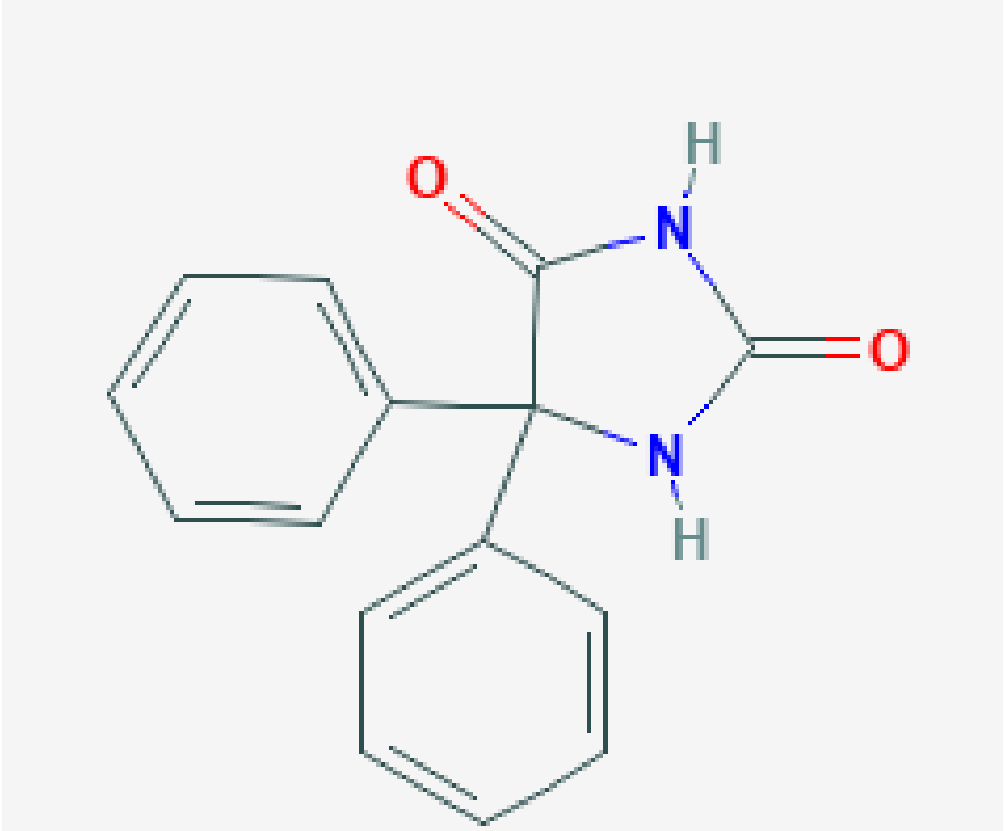


Figure : 1: Structure of Phenytoin

Structure-Activity Relationship

A 5-phenyl or other aromatic substituent appears essential for activity against generalized tonic-clonic seizures. Alkyl substituent in position 5, contribute to sedation, a property absent in phenytoin. The carbon 5 position permits asymmetry, but there appears to be little difference in activity between isomers.

Pharmacological Effects

Central Nervous System

Phenytoin exerts anti-seizure activity without causing general depression of the CNS. In toxic doses, it may produce excitatory signs and at lethal levels it produces decerebrate rigidity. The most significant effect of phenytoin is its ability to modify the pattern of maximal electroshock seizures. The characteristic tonic phase can be abolished completely but the residual clonic seizure may be exaggerated and prolonged. This seizure-modifying action is observed with many other anti-seizure drugs that are effective against generalized tonic-clonic seizures. By contrast, phenytoin does not inhibit clonic seizures evoked by pentylenetetrazol.

Mechanism of Action

Phenytoin reduces the repetitive firing of action potentials evoked by a sustained depolarization of mouse spinal cord neurons maintained *in vitro*. This effect is mediated by a slowing of the rate of recovery of voltage-activated Na^+ channels from inactivation, an action that is both voltage-dependent (greater effect if membrane is depolarized) and use-dependent. These effects of phenytoin are evident at concentrations in the range of therapeutic drug levels in cerebrospinal fluid (CSF) in humans, which correlate with the free (or unbound) concentration of phenytoin in the serum. Within the therapeutic range, the effects on Na^+

channels are selective and no changes of spontaneous activity or responses to iontophoretically applied GABA or glutamate is detected. Whereas if concentration is increased to 5- to 10-fold higher, multiple effects of phenytoin are apparent, including reduction of spontaneous activity and enhancement of responses to GABA; these effects may underlie some of the unwanted toxicity associated with high levels of phenytoin.

Pharmacokinetic Properties

Phenytoin is available in two types of oral formulations that differ in their pharmacokinetics: rapid-release and extended-release forms. Once-daily dosing is possible only with the extended-release formulations and due to differences in dissolution and other formulation-dependent factors, the plasma phenytoin level may change when converting from one formulation to another. Confusion also can arise because different formulations can include either phenytoin or phenytoin sodium. Therefore, comparable doses can be approximated by considering "phenytoin equivalents" but serum level monitoring is also necessary to assure therapeutic safety.

The pharmacokinetic characteristics of phenytoin are influenced markedly by its binding to serum proteins, by the nonlinearity of its elimination kinetics and by its metabolism by hepatic CYPs. Phenytoin is extensively bound (~90%) to serum proteins, mainly to albumin. Small variations in the percentage of phenytoin that is plasma protein bound dramatically affect the absolute amount of free (active) drug leading to increased proportions of free drug, effects of which are evident in the neonate, in patients with hypoalbuminemia and uremic patients. Some agents can compete with phenytoin for binding sites on plasma proteins and increase free phenytoin at the time the new drug is added to the regimen. However, the effect on free phenytoin is only short-lived and usually does not cause clinical complications unless inhibition

of phenytoin metabolism also occurs. For example, Sodium valproate, other group of anti-epileptic drug competes for protein binding sites of phenytoin and inhibits phenytoin's metabolism, resulting in marked and sustained increases in free phenytoin. Measurement of free rather than total phenytoin permits direct assessment of this potential problem in patient management.

Phenytoin is one of the few drugs for which the rate of elimination varies as a function of its concentration (i.e., the rate is nonlinear). The plasma $t_{1/2}$ of phenytoin ranges between 6 and 24 hours at plasma concentrations below 10μg/ml but increases with higher concentrations; as a result, plasma drug concentration increases disproportionately as dosage is increased, even with small adjustments for levels near the therapeutic range.

The majority (95%) of phenytoin is metabolized in the hepatic endoplasmic reticulum by CYP2C9/10 and to a lesser extent CYP2C19. The principal metabolite, a parahydroxy-phenyl derivative, is inactive. Because its metabolism is saturable, other drugs that are metabolized by these enzymes can inhibit the metabolism of phenytoin and increase its plasma concentration. Conversely, the degradation rate of other drugs that are substrates for these enzymes can be inhibited by phenytoin; one such drug is warfarin and addition of phenytoin to a patient receiving warfarin can lead to bleeding disorders. An alternative mechanism of drug interactions arises from phenytoin's ability to induce diverse CYPs; co-administration of phenytoin and medications metabolized by these enzymes can lead to an increased degradation of such medications. Of particular note in this regard are oral contraceptives which are metabolized by CYP3A4; treatment with phenytoin can enhance the metabolism of oral contraceptives and lead to unplanned pregnancy. The potential teratogenic effects of phenytoin underscore the importance of attention to this interaction. Carbamazepine, oxcarbazepine,

phenobarbital, and primidone also can induce CYP3A4 and likewise might increase degradation of oral contraceptives.[19]

The low water solubility of phenytoin hindered its intravenous use and led to production of fosphenytoin, a water-soluble prodrug. Fosphenytoin (CEREBYX, others) is converted into phenytoin by phosphatases in liver and red blood cells with a $t_{1/2}$ of 8-15 minutes. Fosphenytoin is extensively bound (95-99%) to human plasma proteins, primarily albumin. This binding is saturable and fosphenytoin displaces phenytoin from protein-binding sites. Fosphenytoin is useful for adults with partial or generalized seizures when intravenous or intramuscular administration is indicated.

The usual dose of phenytoin for seizures is the extended release formulation (100mg per capsule). Initial dose (in Patients not previously treated with this drug) 1 capsule orally 3 times a day, dosage then adjusted to suit individual requirements.

Maintenance dose: For most adults the satisfactory maintenance dosage will be 1 capsule 3 to 4 times a day, for others an increase up to 2 capsules 3 times a day may be made if necessary.

Chewable tablet has a dosage of 50mg / tablet.

Suspension: (125mg per 5 ml) initial dose (in patients not previously treated with this drug) 5ml orally 3 times daily, dosage then adjusted to suit individual requirements, an increase in up to 25ml orally daily may be made necessary.

PHARMACODYNAMICS:

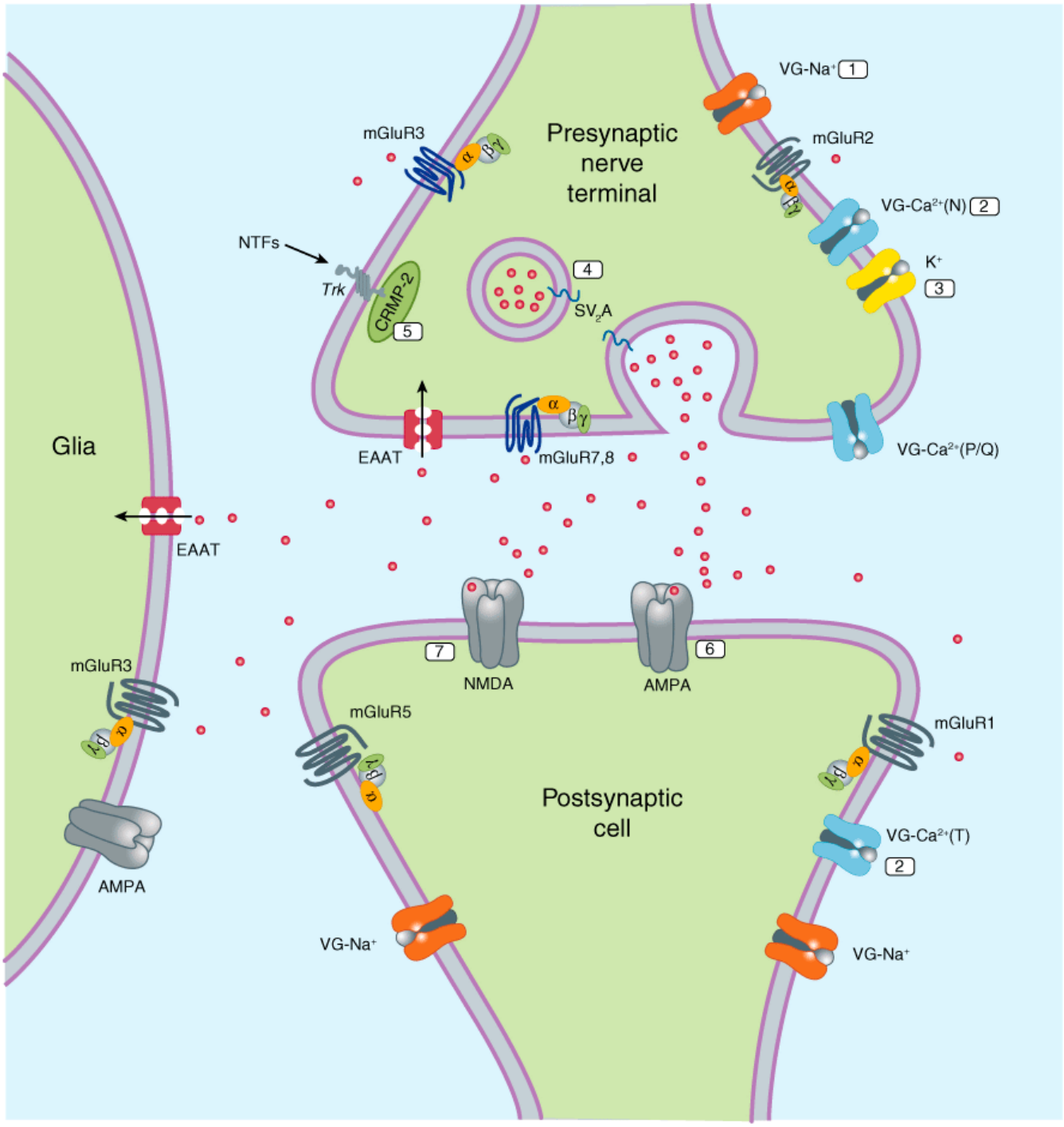


Figure : 2 Mechanism of action of Phenytoin[20]

CENTRAL NERVOUS SYSTEM:

Phenytoin binds to specific site on voltage-dependent sodium channels and is thought to exert its anticonvulsant effect by suppressing the sustained repetitive firing of neurons by inhibiting sodium flux through these voltage dependent channels. Phenytoin stabilizes membranes, protecting the sodium pump in the brain. It limits the development of maximal convulsive activity and reduces the spread of convulsive activity from a discharging focus without influencing the focus itself.

CARDIO VASCULAR SYSTEM:

Phenytoin also stabilises membranes, protecting the sodium pump in the heart. Phenytoin has anti arrhythmic properties similar to those of quinidine or procainamide. Although phenytoin has minimal effect on the electrical excitability of cardiac muscle, it decreases the force of contraction, depresses pacemaker action and improves atrioventricular conduction. It also prolongs the effective refractory period relative to the action potential duration.

ADVERSE DRUG REACTIONS:

The toxic effects of phenytoin depend on the route of administration, the duration of exposure, and the dosage. When fosphenytoin, the water-soluble prodrug, is administered intravenously at an excessive rate in the emergency treatment of status epilepticus, the most notable toxic signs are cardiac arrhythmias with or without hypotension, and/or CNS depression. Although cardiac toxicity occurs more frequently in older patients and in those with known cardiac disease, it also can develop in young, healthy patients. These complications can be minimized by administering fosphenytoin at a rate of < 150 mg of phenytoin sodium

equivalents per minute. Acute oral over dosage results primarily in signs referable to the cerebellum and vestibular system; high doses have been associated with marked cerebellar atrophy.

CNS TOXICITY:

Toxic effects associated with chronic treatment also are primarily dose-related cerebello-vestibular effects but also include other CNS effects. These include nystagmus, ataxia, slurred speech, decreased coordination, and mental confusion. Dizziness, insomnia, transient nervousness, motor twitching, and headache have also been observed. There have also been rare reports of phenytoin induced dyskinesias, including chorea, dystonia, tremor, and asterixis. A predominantly sensory peripheral polyneuropathy has been observed in patients receiving long-term phenytoin therapy.

OTHER TOXICITIES:

Gingival hyperplasia occurs in around 20% of all patients on chronic phenytoin therapy and is probably the most common manifestation of phenytoin toxicity in children and young adolescents. It may be more frequent in those individuals who also develop coarsened facial features. The overgrowth of tissue appears to involve altered collagen metabolism. Toothless portions of the gums are not affected. The condition does not necessarily require withdrawal of medication and can be minimized by good oral hygiene.

A variety of endocrine effects have been reported. Inhibition of release of anti-diuretic hormone (ADH) has been observed in patients with inappropriate ADH secretion. Raised sugar levels in blood and urine appear to be due to inhibition of insulin secretion. Rarely hyperglycemic

hyperosmolar non-ketotic coma complicates IV Phenytoin therapy. Hypertrichosis is seen especially in children. Long term phenytoin therapy sometimes causes hyoertrophy of facial subcutaneous tissue, Hypertrichosis and coarsening of facial features (Phenytoin Facies).[21] Osteomalacia, with hypocalcemia and elevated alkaline phosphatase activity, has been attributed to both altered metabolism of vitamin D and the attendant inhibition of intestinal absorption of Ca^{2+}. Phenytoin also increases the metabolism of vitamin K and reduces the concentration of vitamin K–dependent proteins that are important for normal Ca^{2+} metabolism in bone. This may explain why the osteomalacia is not always ameliorated by the administration of vitamin D.

Hypersensitivity reactions include morbilliform rash in 2-5% of patients. Occasionally more serious skin reactions, including Stevens-Johnson syndrome, Toxic epidermal necrolysis, Fetal hydantoin syndrome, Systemic lupus erythematosus (SLE) and potentially fatal hepatic necrosis have also been documented. Hematological reactions include neutropenia and leukopenia, red-cell aplasia, agranulocytosis, and mild thrombocytopenia also have been encountered. Lymphadenopathy, resembling Hodgkin's disease and malignant lymphoma, is associated with reduced immunoglobulin A (IgA) production. Hypoprothrombinemia and hemorrhage have occurred in the newborns of mothers who received phenytoin during pregnancy; vitamin K is effective treatment or prophylaxis.[19]

Plasma Drug Concentrations

A good correlation usually is observed between the total concentration of phenytoin in plasma and its clinical effect. Thus, control of seizures generally is obtained with total concentrations above 10μg/mL, while toxic effects such as nystagmus develop at total concentrations around 20 μg/mL. Control of seizures generally is obtained with free phenytoin concentrations of 0.75-1.25μg/mL.

Drug Interactions

- Concurrent administration of any drug metabolized by CYP2C9 or CYP2C10 can increase the plasma concentration of phenytoin by decreasing its rate of metabolism ,
- Carbamazepine, which may enhance the metabolism of phenytoin, causes a well-documented decrease in phenytoin concentration. Conversely, phenytoin reduces the concentration of carbamazepine.
- Interaction between phenytoin and phenobarbital is variable.
- Phenytoin being an enzyme inducer, increases the mechanism of corticosteroids, oral contraceptives, doxycycline, rifampicin, theophylline, levodopa, Vitamin D and Vitamin K.
- Enzyme inhibitors like disulfiram, isoniazid, cimetidine, chloramphenicol decrease the metabolism of phenytoin.[22]

Therapeutic Uses

Epilepsy

Phenytoin is effective against complex partial and tonic-clonic but not absence seizures. The use of phenytoin and other agents in the therapy of epilepsies is discussed further at the end of this chapter. Phenytoin preparations differ significantly in bioavailability and rate of absorption. In general, patients should consistently be treated with the same drug from a single manufacturer. However, if it becomes necessary to temporarily switch between products, care should be taken to select a therapeutically equivalent product and patients should be monitored for loss of seizure control or onset of new toxicities.[23]

Other Uses

- Trigeminal and related neuralgias
- Cardiac arrhythmias- Refractory Ventricular Arrhythmia

PIRACETAM

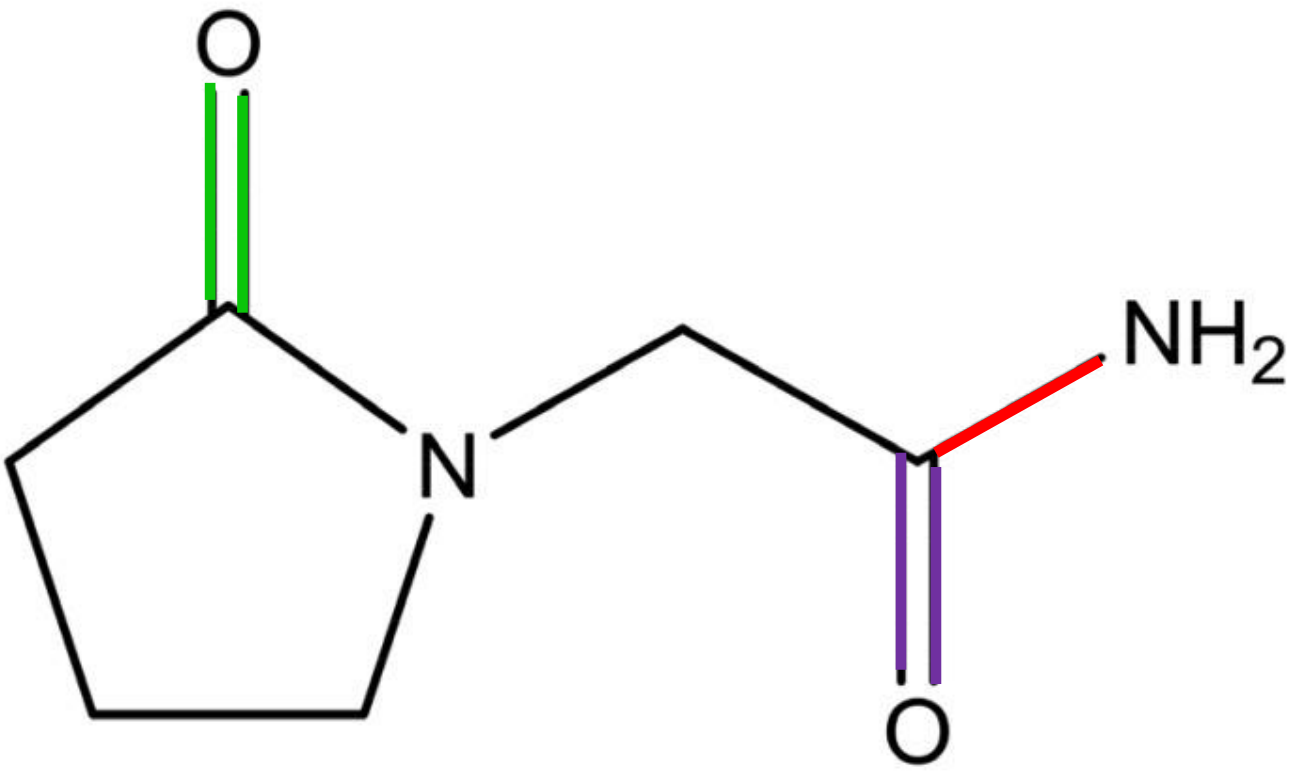


Figure: 3: Structure of Piracetam

PIRACETAM

Piracetam was developed in 1960s in Belgium as a ***'smart drug'***. It is a recurring plagiaristic form of GABA, also known as aminobutyric acid. Since its discovery and usefulness detection, piracetam has been used as a nootropic agent for the enhancement of memory in human beings. It did not produce harmful and toxic effects. Austria, Germany and Switzerland, it is indicated in stroke and mild cognitive impairment. Levetiracetam, another commonly used nootropic agent was developed in 1999 to cure epilepsy. It has been licensed since 1999 in USA and 2000 in Switzerland.

Piracetam is the prototype of the nootropic agents, a class of drugs activating brain integrative mechanisms and improving learning and memory processes. Other nootropic agents include Rolziracetam, Aniracetam, Pramiracetam and Oxiracetam.

It is a nootropic with low contagiousness and insolubility levels and incurs a small number of side effects. Its effectiveness in the treatment of ischemia, cognitive impairment, stroke and dementia is acknowledged widely. It also contributes to the cognition enhancement of dyslexic and dyspraxic children. Its usefulness is equally apparent in Down syndrome patients whereby piracetam is employed to slow down the rapid aging of the brain. It also contributes as a modulator of brain metabolism, neuro-protection and neuro-plasticity. Piracetam is also known to have anti-seizure or antiepileptic effects.

Characteristics of an ideal Nootropic Agent:

a) It should protect the brain from physical and chemical injuries.
b) It should enhance the memory and learning processes.
c) It should act against those agents which may impair memory and behavior.
d) It should not produce harmful and toxic effects.

Pharmacological Properties of Nootropics:

Nootropics influence cholinergic function. These drugs enhance the synthesis of acetylcholine by increasing high affinity choline uptake at muscarinic and nicotinic receptors. As the human beings age advances, a depletion of acetylcholine receptors has been observed. Piracetam acts by enhancing the number of acetylcholine receptors in frontal region of the brain. So, ultimately it increases the level of acetylcholine in the brain by 30-40 %.

In case of dementia or Alzheimer's disease the carbohydrate metabolism of our brain is declined. Brain cells produce their own ATP from glucose and sugar as they cannot get it from any other source.

Piracetam acts by activating adenylate kinase enzyme that is responsible for the conversion of ADP in to ATP and AMP. So, this compensates the deficiency of ATP in the brain cells. This may ultimately serve a crucial role in preventing and curing dementia by recovering the oxygen and energy demand in the brain

- It also elevate the cerebral blood supply, oxygen supply, glucose metabolism rate in human brain functioning which was impaired from a long period like in the case of multi-infarct dementia, senile dementia, pseudo dementia, poor brain blood flow.
- It also possesses antithrombotic activity in vivo. It has been shown to normalize platelet aggregation in patients with acute stroke, transient ischemic attacks and diabetes mellitus.[24]

MECHANISM OF ACTION OF PIRACETAM:

The Membrane Hypothesis:

Piracetam not only affects the membrane fluidity of the brain but also the membrane of blood platelets. Piracetam acts by restoring the membrane fluidity. Membrane fluidity is crucial for the regulation of membrane transport, enzyme activity, chemical secretion, receptor binding nd stimulation. It has been reported that piracetam interacts with the cell membranes and prevented the appearance of alcohol related changes in a synthetic phosphatylcholine monolayer.

It was observed that amyloid peptide gets aggregated on the neuronal membranes and causes lipid disorganization within the cell membranes. Piracetam reduces the destabilizing effects of the amyloid peptide. This could happen as a result when piracetam interacts with phospholipid head groups of the cell membranes.

Piracetam also plays an important role in energy metabolism by enhancing the utilization of oxygen in the brain and enhancing the cell permeability and also the permeability of mitochondrial membranes to the intermediate products of Krebs's cycle and acts as an antioxidant/neurotonic. It also enhances the number of acetylcholine receptors. The agents belonging to piracetam, oxiracetam and aniracetam stimulate AMPA type glutamate receptors which enhance the number of receptor binding sites for AMPA and calcium uptake. [21]

PIM (2-oxo-1-pyrolidone acetamide)-a nootropic has been shown to be an effective anti-myoclonic agent. It has been shown to have a specific anti-amnesic activity. In addition, it has demonstrated a protective effect against pentylenetetrazole kindling-induced neuronal loss and learning deficit. However, it lacks anticonvulsant activity in the Maximal Electroshock Model (MES). Convincing neuroprotective functions have also been shown experimentally. Thus it

would be worthwhile to assess the use of Piracetam along with Phenytoin on seizure and cognitive functions. The central cholinergic system plays an important role in learning and memory. Phenytoin is known to reduce hippocampal ACh concentration. In view of this we also studied the effect of this combination on the brain cholinergic system. Since the majority of anti-epileptic drugs including PHT are known to impair motor performance, the study also evaluated this combination on motor function. [22]

This drug is a cyclic GABA derivative has no GABA like activity and has been called Nootropic meaning a drug selectively improve efficiency of higher telencephalic integrative activities.

Nootropic drugs are widely used for treating neurological disorders like:

(i) cognition/memory;

(ii) epilepsy and seizure;

(iii) neurodegenerative diseases;

(iv) stroke /ischemia;

(v) stress and anxiety.

Piracetam is not a vasodilator, does not affect total/ regional blood flow but may reduce the blood viscosity. In India and some other countries it has been promoted for cognition impairment and dementia in the elderly as well as for mental retardation in age group over 30 years. It may benefit in cognitive disorders of cerebrovascular and traumatic origin. In the United Kingdom (UK), it is approved for adjunctive treatment of cortical myoclonus, but is not recommended for children.

DRUG INTERACTIONS:

Piracetam is not metabolized in the liver. It is not plasma protein bound. So, there are very less chances of drug–drug interactions. The drug has been observed to increase the anticonvulsant activity of carbamazepine. But, there are no interactions of any other drugs with piracetam. [25]

CONTRAINDICATIONS:

Piracetam should not be administered in patients with renal disorder since it eliminated via kidneys and care should be taken in cases of renal insufficiency and it is strictly contraindicated in patients with end-stage renal disease. It is also contraindicated in patients with cerebral hemorrhage. It cannot be safely used in pregnant and lactating women as no study on human is done although no risk to fetus has been observed yet.

DISEASE AFFECTING COGNITIVE IMPAIRMENT:

- **Neurodegenerative disorders:**
    - Alzheimer's Disease,
    - Dementia With Lewy Bodies,
    - Parkinson's Disease,
    - Huntington's Disease.
- **Vascular:**
    - Infarction,
    - Binswanger's' Disease.
- **Neurological Disease:**
    - Multiple Sclerosis,
    - Normal Pressure Hydrocephalus,
- **Brain Tumour**

- **Nutritional Disorders:**
    - Vitamin B12 Deficiency,
    - Thiamine Deficiency,
    - Niacin Deficiency.
- **Endocrine:**
    - Hypothyroidism,
    - Hypercalcemia
- **Infectious:**
    - Human Immunodeficiency Disease,
    - Prion Disease,
    - Neurosyphilis.
- **Metabolic:**
    - Hepatic & Renal Insuffiency,
    - Wilson's Disease,
    - Metachromatic Leukodystrophy.
- **Drugs & Toxins Affecting Cognitive Impairment:**
    - Exposure to Alcohol,
    - Heavy Metals,
    - Anticholinergic Medications,
    - Carbon monoxide,
    - Irradiation.[6]

CELASTRUS PANICULATUS:

English Name: Intellect Tree, Black-Oil Plant, Climbing Staff Tree

Hindi Name: Kondgaidh, ,Malkangani, Sankhu

Sanskrit Name: Jyotishmati, Jyotishka, Katabhi, Kanguni

- **Kingdom:** PLANTAE
- **Sub-Kingdom:** VIRIDIPLANTAE
- **Infra Kingdom:** STREPTOPHYTA (land plants)
- **Super Division:** EMBRYOPHYTA
- **Division:** TRACHEOPHYTA (TRACHEOPHYTES or Vascular Plants)
- **Sub Division:** SPERMATOPHYTINA (SPERMATOPHYTES or Seed Plants)
- **Class:** MAGNOLIOPSIDA
- **Super Order:** Rosanae
- **Order:** Celastrales
- **Family:** Celastraceae – Bittersweet
- **Genus:** Celastrus
- **Species:** Celastrus *Paniculatus* or C. *Paniculatus*

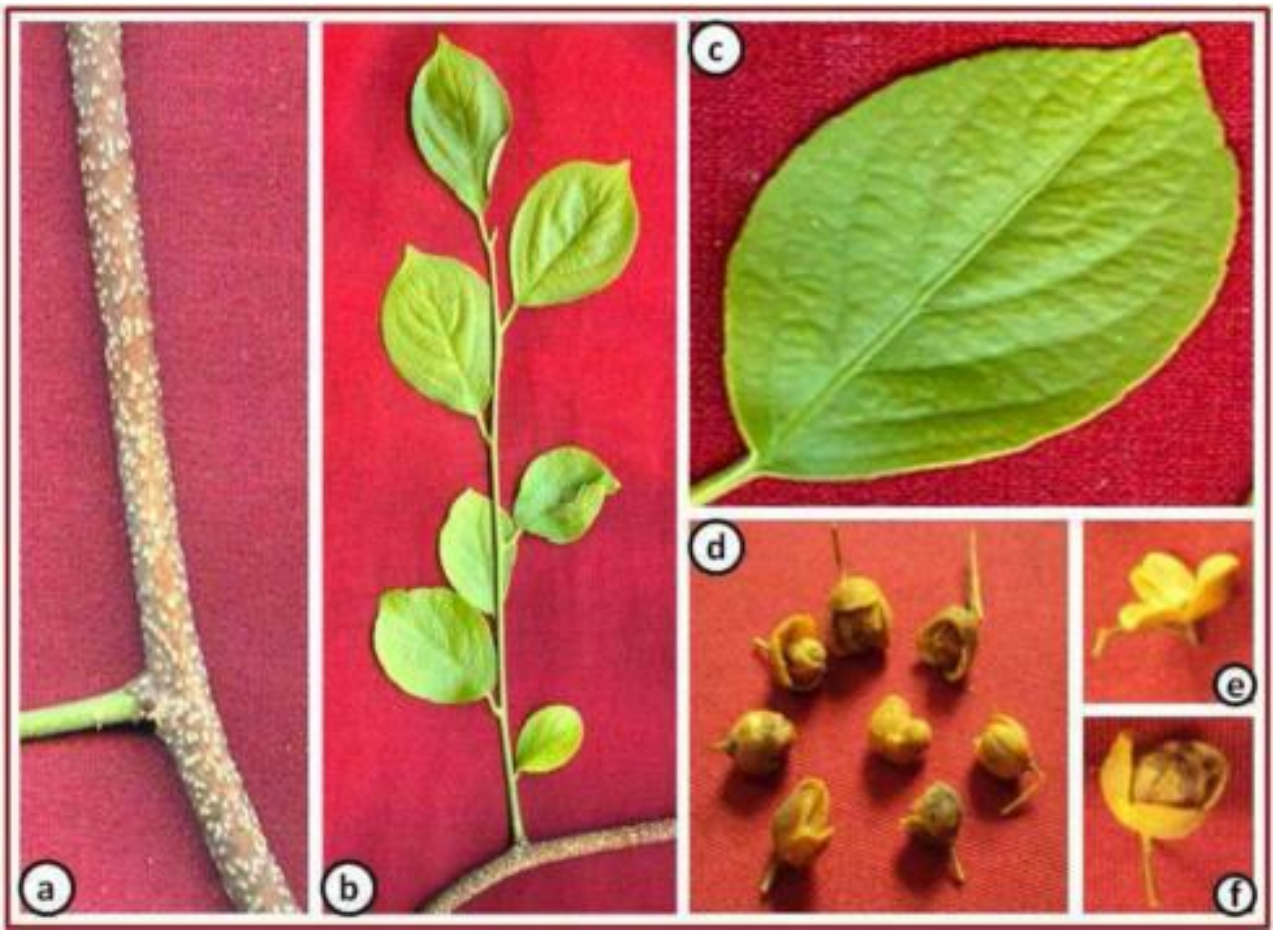


Figure : 4[26]

a) Stem: Reddish brown stem covered with small elongated white lenticels

b) Leaves: Alternately arranged

c) Ovate leaf

d) Capsules: Orange colored with 3-6 seeds inside

e) Dehisced capsule without seeds

f) Seeds: Single capsule showing seeds enclosed by an orange-red aril.

This climbing shrub grows throughout India at elevations up to 1,800 m (5,900 ft).*C. paniculatus* is a deciduous vine with stems up to 10 cm (3.9 in) in diameter and 6 m (20 ft) long with rough, pale brown exfoliating bark covered densely with small, elongated lenticles. The leaves are simple, broad, and oval, obovate or elliptic in shape, with toothed margins. Medicinal plants with their intraspecific variation represent a chemical and medicinal goldmine as is evident from the strong traditions of natural drug use. *Celastrus paniculatus* wild, mentioned in Ayurveda as "Tree of life", was in use from time immemorial to treat brain related disorders and to enhance learning and memory. The Jyotishmati oil extracted from the seeds of *C. paniculatus* is known to have effect on Central Nervous System. It is used to treat acute and chronic immobilization stress. The oil obtained from the seeds possesses sedative and anticonvulsant properties. Seed oil has been found to be beneficial to psychiatric patients; and increase the intelligence quotient of mentally retarded children. [27]

9.1: TAXONOMY OF CELASTRUS PANICULATUS:

Botanical Description:

Celastrus Paniculatus is the botanical name of Indian herb known as Jyotishmati and Malkangani. It belongs with bittersweet family named Celastraceae.

Figure: 5

Celastrus Paniculatus plant In Dhanalakshmi Srinivasan Medical College- **Herbal Garden.**

Figure: 6 Celastrus paniculatus seeds, oil, leaves.[26]

MEDICINAL PARTS:

Celastrus paniculatus Seeds and its oil are mainly used in ayurvedic medicines. Leaves are also used for De-addiction. Generally, leaf juice is beneficial for treating opium addiction.

PHYTOCHEMISTRY: (Chemical Composition)

Celastrus paniculatus Seeds contains around 30% oil content in which following alkaloids are present.

- ***CELAPAGIN***
- ***CELAPANIGIN***
- ***CELAPANIN***
- ***CELASTRINE***
- ***PANICULATINE***

The classes of molecules most predominant in this plant appear to be:

- Sequesterpene alkaloids and Polyalcohols.

Overall, the phytochemical evaluation reveals the presence of:

- Carbohydrates
- Fixed oil
- Glycosides
- Cumarines
- Tannins
- Flavonoids
- Saponins
- Steroids
- Triterpenoids

which have been claimed to be responsible for its therapeutic uses.

The trace elements found in Celastrus paniculatus and known to be essential for humans and unquestionably associated with deficiency symptoms include:

- Chromium
- Copper
- Iodine
- Iron
- Manganese
- Molybdenum
- Selenium
- Zinc.

CNS PROPERTIES: Celastrus paniculatus has well known Nootropic property. It is also used for improving intellect, memory loss & dementia and neurodegenerative diseases.

OTHER PROPERTIES:

- Analgesic
- Nervine Stimulant
- Digestive Stimulant
- Carminative
- Cardiac Stimulant
- Diuretic
- Aphrodisiac
- Erectogenic
- Anti-rheumatic
- Anti-arthritic
- Anti-inflammatory
- Anti-nociceptive
- Emmenagogue
- Diaphoretic
- Febrifuge
- Thermogenic
- Intellect promoting
- Anti-proliferative.

THERAPEUTIC INDICATIONS:

Celastrus paniculatus (Malkangani) is helpful in following health conditions: Memory Loss, Cognitive & Concentration Problems, Rheumatic arthritis, Insomnia, Gouty Arthritis, Opium addiction, Opium Poisoning , Bradycardia, Impotence, Facial paralysis, Sciatica, Secondary Amenorrhea.

Celastrus Paniculatus benefits are: Seeds are used in powder form and taken with milk. Its seeds have effects on brain, mind, nerves, joints and bones, Improves Intellect.

The plants usage has been extensively researched with promising results to treat neurodegenerative diseases such as Alzheimer's. Mice receiving Celastrus paniculatus showed significant memory enhancement.

Apart from neurological properties, the alcoholic extracts of Celastrus paniculatus seeds (AlcE) possess significant antinociceptive and antiinflammatory activity in-vivo. Histological studies show less cholesterol deposits in the aorta of animals fed with seed extract of Celastrus paniculatus compared to the induced hypercholesterolemic animals not given Celastrus paniculatus supplement. It helps in reducing the activites of HMG-CoA reductase, glucose 6-phosphate dehydrogenase and malate dehydrogenase which are associated with cholesterol synthesis.

1. **Memory loss &Dementia:** It is memory booster herb, which improves recall and retention span & as brain tonic for increasing memory. Seeds and oil both are effective in forgetfulness and memory disorders. Malkangani should be used in dosage of 5 to 15 drops in milk. Seeds can be taken in powdered form in dosage of 1 gram with milk. In dementia, it stops its progress by preventing cell damage in the brain. It increases glutathione and catalase levels and decreases malondialdehyde (reactive species) in the brain, which might be responsible for its antioxidant, neuroprotective and cognitive-enhancing actions.

2. **Neurodegenerative Diseases:** The neuroprotective effects might be due to its antioxidant action, which helps reducing oxidative damage of the neurons. MEDHYA (Nootropic) effect of Celastrus paniculatus is well-established in ayurveda and it is widely used for this purpose. It induces alertness, improves concentration, reduces rate of cell death of neurons, improves ability of thinking and reasoning and helps tackling stress disorders.

3. **Atherosclerosis & High Cholesterol:** It has anti-lipidemic effects and reduces atherogenic index. Its use reduces total cholesterol and anti-inflammatory property helps reducing inflammation of blood vessels, which helps stopping or slowing down the progress of atherosclerotic lesions. It significantly lowers the elevated cholesterol and LDL cholesterol, which helps preventing cardiac diseases and atherosclerosis.

4. **Fatty Liver:** Celastrus paniculatus seeds decrease fat deposition in liver. This action can help treating people with fatty liver syndrome or enlarged liver.

5. **Osteoarthritis:** Celastrus paniculatus seeds possess significant anti-nociceptive characteristic and anti-inflammatory property, which helps reducing joint inflammation and joint pain. In osteoarthritis, 1 grams seed powder is recommended and taken with cow's milk. It has thermogenic action, which also induces heat sensation in the body, so its use is suggestible in winters.

6. **Impotence:** Seeds have potent aphrodisiac, erectogenic and stimulant action. For improving male performance, it should be used along with milk.

7. **Insomnia:** It has anti-stress and calming effect, which helps inducing sound sleep. In ayurveda, the following Jyotishmati combination is used for insomnia.

INGREDIENTS PROPORTION

- Jyotishmati (Malkangani) Seeds 25%,
- Jatamansi 25%
- Misri (Crystallized Sugar) 50%

½ teaspoon of Malkangani mixture should be taken with milk 2 hours before bedtime. If problem is severe, then sarpagandha powder can also be added in this mixture.

8. **Opium Addiction & Opium Poisoning:** It acts as potent antidote for opium and can help people in opium addiction. It is unique ayurvedic medicine, which helps in opiate withdrawal, opiate de-addiction, but you should also consider other supporting medicines according to the symptoms of opiate withdrawal. The symptoms like agitation, increased tearing, anxiety, muscle aches, sleeplessness and runny nose are well controlled with Celastrus Paniculatus.

9. **Dysmenorrhea:** The roasted seeds of Malkangani (Celastrus Paniculatus) along with China rose flower powder are used to promote easier menstruation and reduce menstrual cramps. It is good ayurvedic herbal remedy for primary and secondary dysmenorrhea.

10. **Beriberi:** Jyotishmati Oil contains a good amount of Vitamin B1 (thiamine). Beriberi occurs due to nutritional deficiency of VitaminB1 (thiamine). Therefore, it is used in dosage of 15 drops thrice a day added in the milk.

11. **Knee Pain:** The seeds are traditionally used for knee pain treatment. It reduces pain, joint crepitation, inflammation and stiffness. It is best to use during the winter season it has very hot potency. Its optimum dosage (500 to 1000 mg twice daily) can be used during winters. It has very hot potency and one can experience excess heat sensation in the body. Its use in summers, then dosage should be reduced to 250mg twice daily. With high dosage, some patients may experience vertigo after taking Malkangani in summers. It can be continued for 3 months.

Dosage & Administration:

For Seeds: The general dosage of Celastrus paniculatus (Malkangani or Jyotishmati) seeds is as follows.

Children: 10 mg per Kg weight, but dosage of Malkangani seeds should not exceed from 500 mg , Adults 500 mg to 2 grams

Pregnancy: ***CONTRAINDICATED***

Maximum Possible Dosage 4 grams Per Day (in divided doses)

Figure 7: Commercially available (Pure Malkangani oil)

The general dosage of Celastrus Paniculatus (Malkangani or Jyotishmati) Oil is as follows.

- Children 1 to 5 drops
- Adults 5 to 15 drops
- Pregnancy: ***CONTRAINDICATED***

Maximum Possible Dosage 45 drops Per Day (in divided doses)

Safety Profile: Generally, low dosage (around 500 mg) of Celastrus Paniculatus seeds is well tolerated and likely safe in all kinds of patients. According to ayurveda, Celastrus paniculatus (Jyotishmati or Malkangani) has very hot potency and acrid in nature, which is likely to increase Pitta in the body. Therefore, it is recommended in patients with Vata and Kapha body type or having Vata and Kapha dominant symptoms.

Side Effects: Inappropriate dosage or wrong use in Pitta dominant people of Celastrus Paniculatus seeds or oil can lead to following:

- Restlessness
- Giddiness
- Heat sensation
- Burning sensation
- Excessive sweating

Pregnancy & Lactation:

Celastrus paniculatus (Jyotishmati/Malkangani) can also act as abortifacient and may lead to miscarriage in pregnant women, Hence contraindicated, if you are trying to conceive, during pregnancy and postpartum period.

Contraindications:

- Hyperacidity
- Bleeding disorders
- Pregnancy
- When trying to conceive
- Postpartum period
- 30 days before and after surgery
- Uterine heavy bleeding
- Heavy menstruation.[28]

WHY ANIMAL MODELS FOR THIS STUDY? :

In recent years, the practice of using animals for biomedical research has come under severe criticism by animal protection and animal rights. Use of animal in research is a moral issue because animals are harmed in experimentation, from such things as confinement, fear [from handling], pain and early death. Those against, contend that the benefit to humans does not justify the harm to animals. Many people also believe that animals are inferior to humans and very different from them, hence results from animals cannot be applied to humans. Those in favor of animal testing argue that experiments on animals are necessary to advance medical and biological knowledge.

Animal models of cognitive impairment are critically important for determining the neural bases of learning, memory, and attention. These cognitive functions are the result of complex interactions of a variety of neural systems and thus cannot be well studied by simple *in vitro* models. Animal models of cognitive impairment are critical for determining the neural basis of cognitive function as well as for testing the efficacy of potential therapeutic drugs and the neurocognitive toxicity of environmental contaminants and drugs of abuse. A variety of models have used classic monkey, rat, and mouse models. Newer, non-mammalian complementary models with fish, flies, and flatworms are being developed. These will play an important role in both high-throughput screening of potential toxic or therapeutic compounds and in the determination of the neuromolecular bases of cognitive function.[29]

Mice are becoming increasingly valuable in efforts to determine the molecular bases of cognitive function. In addition, genetically manipulated mice are increasingly being used in the development of models for new drug development. For these uses it is important to devise a valid, reliable, and quick battery of tests to determine cognitive function in mice. Application of transgenic mice to problems presented by amyloid deposition with aging is an especially promising forum for the development of new treatments for Alzheimer's disease and other aging related cognitive impairments. Pharmacological models have shown that acetylcholine plays key roles in the neural bases of cognitive functions. Cholinergic-receptor knockout mice are being used to good effect in determining the role of various aspects of the cholinergic systems in cognitive function.

Animal models can quite well simulate specific syndromes of cognitive impairment where the inciting faction is well known. Prime examples of this approach include studies of aging and neurotrauma. Aging studies, especially with long-lived species such as monkeys, readily demonstrate aging-induced cognitive impairment and serve as a fine basis for developing new treatments and novel drugs. Neurotrauma causes cognitive impairment in animal models in quite similar ways as in humans. The specific mechanisms underlying such impairment and the therapeutic treatments for it can be well studied in animal models.

New non-mammalian models of cognitive impairment are being developed. These models are sometimes called alternative models but are better termed complementary models, because they are best used not in place of mammalian models but to complement them. Mammalian and non-mammalian models each have their own sets of advantages and disadvantages, which can be used in a mutually complementary fashion in a strategy of research advancement. Mammals have a high degree of neuroanatomic similarity to humans, but they are generally expensive and time consuming models to use.

Animal models of cognitive impairment play crucial roles in the characterization of toxicants that cause cognitive dysfunction and the identification of potential new drugs for treating cognitive dysfunction, as well as providing critical insight into the neural bases of cognitive function and dysfunction. There are a variety of important issues specific to each model and in general across models that must be considered if these models are to be used productively.[25]

NEED FOR ANIMAL EXPERIMENTS:

Animal studies are mandatory because at the current level for studying the pathogenesis of different disease, to undertake drug trials, vaccines to alleviate suffering in the humans it is necessary. In vitro alternate methods cannot replace animal experimentation totally, but can work only as adjuncts and reduce the number of animals to the extent possible. To achieve an effective anti-convulsive therapy, it is expected to attain complete seizure control without interrupting any cognitive effects caused by

phenytoin and the cognitive deficits can be improved with Celastrus paniculatus herbal plant as this study is focused.

There are various tests to assess the learning and memory in animal models – rats and mice.

They are:

1) Pole climbing apparatus test
2) Spontaneous alteration behavior on a plus maze test
3) Morris water maze test
4) Radial arm maze test
5) Hebb's William maze test
6) Barnes maze test.
7) T – maze test
8) Object recognition tests

Among these above test pole climbing test and 8 radial arm maze tests has been taken for our study to assess the learning and memory in mice animal models.

NEURO TRANSMITTERS IN CENTRAL NERVOUS SYSTEM RESEARCH STUDIES:

Neurotransmitters are chemicals that travel across the synapse and allow communication between neurons throughout the brain and body. They are the chemical messengers in humans and are associated with several CNS disorders .Plant drugs can be used as agonist / antagonist/ modulators to neurotransmitters to treat Alzheimer's disease, Parkinson's disease etc.

The following are the certain neurotransmitters and its functions:

1. **Acetylcholine:** It is an excitatory neurotransmitter in CNS involved in wakefulness, attentiveness, learning and memory, anger, aggression sexuality and thirst.

2. **Dopamine:** It also an excitatory neurotransmitter in CNS involved in control and posture. If the dopamine is elevated or low there will be inattention, forgetfulness.

3. **Nor- epinephrine:** It helps to make epinephrine, Elevated levels causes anxiety and mood dampening effects and low levels causes decreased focus and sleep cycle problems.

4. **Epinephrine:** It is an excitatory neurotransmitter in CNS, will be elevated when Attention deficit hyperactivity disorder symptoms are present. Long term stress and insomnia causes its levels to be depleted.

5. **Serotonin:** It is a monoaminergic neurotransmitter, 5HT1A is found to be involved in psychiatric disorders like depression, schizophrenia and anxiety and becomes the target of these diseases.[30]

NEED FOR NEWER MOLECULES FOR COGNITION ENHANCEMENT:

Since phenytoin is the first line choice for seizure disorder in allopathic medicine and on long term therapy leads to impaired cognition, so it is mandatory to have a drug for seizure without disturbing cognition, there is a need for therapy in other modes of branches of medicine, we found that there are herbal drugs which is found to enhance cognition. Since there are fewer studies on it, so we decided to carry our work with herbal drug.

MATERIAL AND METHODS:

ANIMALS:

- Male Swiss Albino Mice of 30–45 g weight 72 Nos. were purchased from King's Institute Guindy, Chennai Tamilnadu and transported to Animal house of DSMCH
- Diet - standard pellets for mice - purchased in King's institute Guindy.
- The animals were housed in polypropylene cages (22.5 x 35.5 x l5cm) with false mesh to avoid coprophagy and controlled temperature (25 ± 2°C), humidity (50-55%) and light (12h-light-dark cycle) environment.
- Weight will be measured at baseline.
- The mice were allowed to acclimatize for these conditions for one week and the animals were permitted for free access to a standard pellet diet and tap water.

DRUGS:

1. Dilantin suspension product of Pfizer Inc,
2. Nootropil syrup product of UCB laboratories,
3. Collection of Celastrus paniculatus seed oil: The oil was acquired from Deve Herbes New Delhi, from reputed herbal pharmaceutical company by placing an order through Amazon online purchasing of 50 ml bottle named as pure Malkangani oil.

CHEMICALS:

- Acetylthiocholine iodide,
- 5,5- dithiobis (2- nitrobenzoic acid) [DTNB],
- 1% Tween-20(solubilising agent)
- Dimethyl sulphoxide (solvent),
- Ophthaldialdehyde (OPT) reagent: (20 mg in 100 ml conc. HCl)

Purchased from "SRL DIAGNOSTICS CHENNAI.TAMILNADU".

- 0.4M HCl, Sodium acetate buffer (pH 6.9)
- 5M NaOH,
- 0.1M Iodine solution (in Ethanol):
- Na2 SO3 sol. ((0.5 g Na SO in 2 ml H O + 18 ml 5 M NaOH),
- 10M Acetic acid

Obtained from the Department of Biochemistry, Dhanalakshmi Srinivasan Medical College & Hospital.

Drugs and Dosing Schedules

- Phenytoin marketed as "Dilantin" in the form of suspension was administered orally in doses of 8, 12, and 22 mg/kg, 2 h prior to each observation.
- Piracetam, the nootropic standard ("Nootropil" syrup) was given orally in a volume of 125,250, and 500 mg/kg body weight 1 h prior to each experiment.

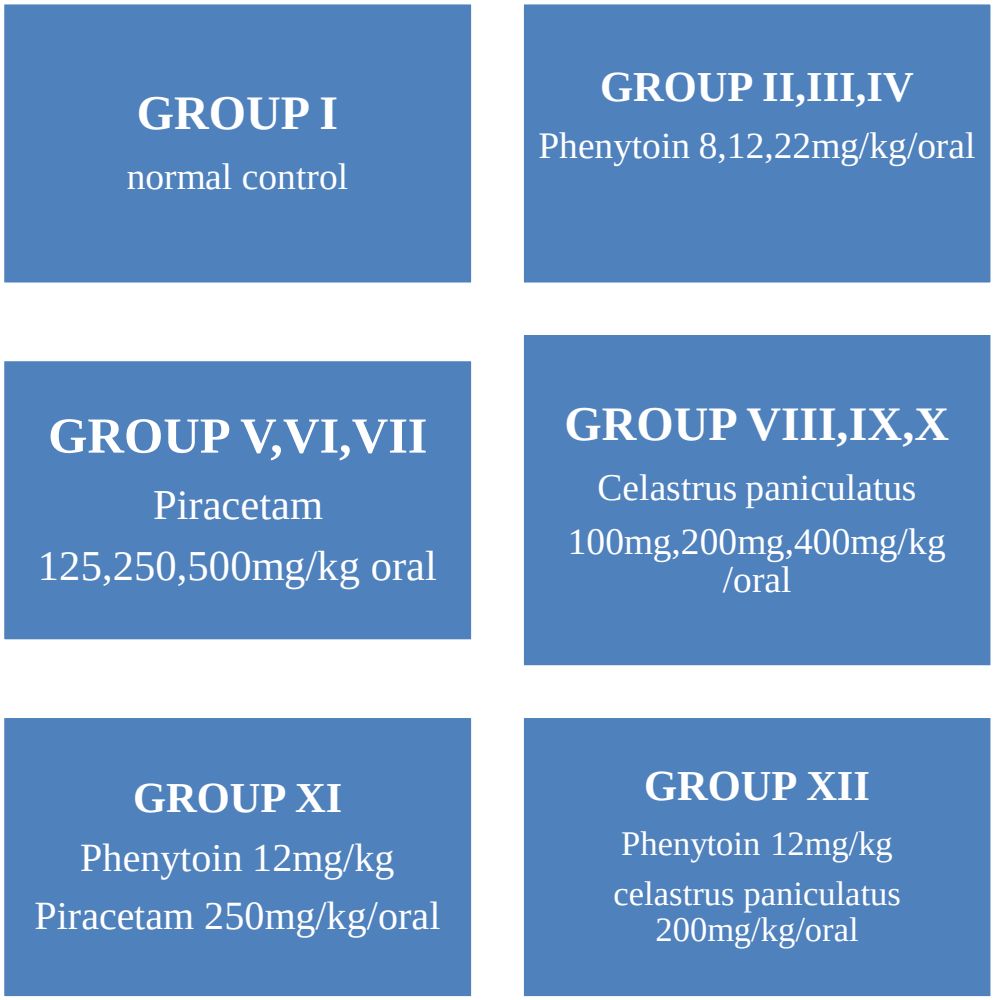
- Celastrus paniculatus, the Experimental drug was given orally with doses 100,200, 400mg kg body weight 1 h prior to each experiment.
- Control groups were administered standard pellet diet and tap water (10 ml/kg).

All observations were made on the day 22 after 2 hours of phenytoin and 1 hour of Piracetam, Celastrus paniculatus administration. During these studies, drugs were administered between 10 am and 12 pm.

GROUPING OF ANIMALS:

Animals were divided into 12 groups containing six animals in each group. Group I served as Normal control. Group II, III, and IV received Phenytoin in dose of 8mg/kg 12mg/kg and 22 mg/kg (i.e): 0.32mg, 0.48mg, 0.88mg. Group V, VI, and VII received Piracetam in dose of 125mg/kg 250mg/kg, and 500mg/kg (i.e): 5mg, 10mg, 20 mg. Group VIII, IX, and X received Celastrus paniculatus oil in the dose of 100mg/kg 200mg/kg and 400 mg/kg (i.e) 4mg, 8mg, 16mg. Group XI received Phenytoin (12 mg/kg) and Piracetam (250 mg/kg) (i.e.) 0.48mg, 10mg while Group XII received Phenytoin (12 mg/kg) and Celastrus paniculatus (200 mg/kg) (i.e): 0.48mg, 8mg respectively.

After the quarantine period the animals will be grouped as follows:



[The submaximal dose of the drugs was selected for groups XI &XII]

After purchasing, commercially available *Celastrus paniculatus* seed oil was emulsified with 1% Tween-20 (solubilising agent) and Dimethyl sulphoxide (solvent). Three different doses (100 mg, 200 mg and 400 mg / kg of body weight) of the seed oil was given orally to the grouped animals for 21days.

ETHICAL APPROVAL:

The experimental protocol was approved by the Institutional Animal Ethics Committee of Dhanalakshmi Srinivasan Medical College& Hospital, Perambalur, Tamilnadu and the study was constituted as per the rules of the committee for the Purpose of Control and Supervision of Experiments on Animals, India and the guidelines of Institutional Animal Ethics Committee (IAEC).

EXPERIMENTAL PROCEDURE:

The animals obtained as mentioned above was transported through well-equipped transporting climate controlled vehicle with standard polypropylene animal cages and filter top appropriately covered to avoid drawing attention to animals without inhibiting airflow.

The mouse were received in the quarantine room and acclimatized for a week with free access to standard pellet diet and tap water and monitored for its wellbeing and reduction in introduction of pathogens into an established colony.

All animals were observed for signs of illness, injury or abnormal behavior by animal house-incharge person. Any abnormality was monitored to ensure timely veterinary medical care.

TOXICITY STUDIES:

Acute and chronic toxicity studies were already done for this Celastrus paniculatus oil in various studies. Hence to reduce the death of small animals- mice, these studies were not performed and the doses were followed accordingly.[27]

They were placed inside polypropylene cages with false mesh to avoid coprophagy with free access to standard pellet and tap water. The weight of the individual animals was measured.

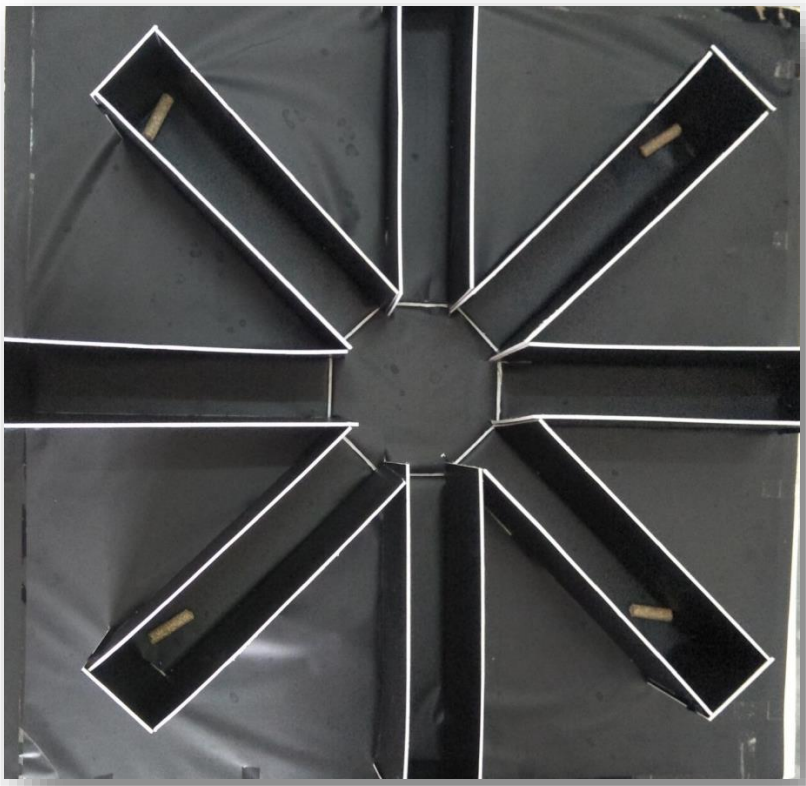
The animals set to be trained and evaluated respectively as follows:

- Three independent assessors were training each animal for 5 minutes for 3days with interval led trials for each experimental methods and was allowed for free access to a standard pellet diet and tap water.

Acute study:

On the 1st day the drugs were given between 10-12 am, that is phenytoin 8mg/kg, 12mg/kg, 22mg/kg 2hr prior to observation and piracetam 125mg/kg, 250mg/kg, 500mg/kg, celastrus paniculatus 100mg/kg, 200mg/kg, 400mg/kg 1hr before observation orally which was followed by experimental methods and findings were validated accordingly.

1. RADIAL ARM MAZE APPARATUS TEST:



8 Radial arm maze apparatus.

The eight arm radial maze apparatus for mice consisted of an equally spaced arms (30 x 6 x 15 cm) radiating from an octagonal central platform**.** Two types of memory assessed are reference memory and working memory.

The animals were kept in restricted diet to maintain a bodyweight of 85%.The animals are subjected to trials individually for 5 minutes per day for two days to explore the maze. Before the commencement of the behavioral assessment, all groups of mice were semi-starved over a period of 48 hrs in order to motivate them towards food reward to acclimatize the mice.

Partially baited task*:* Here, four of the eight arms were baited and the mice were trained to choose only the baited arms. This task permits discerning of reference memory and working memory components of spatial memory.

An entry into an unbaited arm was regarded as a reference memory error and any re-entry either to a baited or unbaited arm was considered as a working memory error. The maze should be cleaned with 70% ethanol and four of the arms (2, 3, 6 and 8) were baited with food reinforcement. The mice were placed in the centre of the octagon and were allowed a free choice. An arm choice was recorded when a mice eats a bait or reached the end of an arm.

The maze arms were not rebaited, so only the first entry into the baited arm was recorded as a correct choice. The trial continued until the mice entered all the four baited arms or 5 minutes had elapsed. After the end of the trial, the mice were returned to the home cages and subjected to second trial after an inter-trial interval of l hour. Training was continued till the mice attained the criteria of 80% correct choice.

Data taken from 4 trials was recorded and represented as blocks and then analyzed for percent correct choice, reference and working memory errors. Percent correct choice was calculated by dividing the number of correct entries by the total number of entries and multiplying by 100. An entry into an unbaited arm was considered a reference memory error (RME) and any re-entry was considered as a working memory error (WME).

2. POLE CLIMBING APPARATUS TEST:

Cook's pole climbing apparatus.

Cook's Pole Climbing Apparatus use to study cognitive function, mainly a response to conditioned stimuli during learning & its retention. The apparatus has an experimental chamber (25 × 25 × 25 cm) with the floor grid in a soundproof enclosure.

Scrambled shock (6mA) is delivered to the grid floor of the chamber composed of stainless steel rods. A pole of 2.5 cm in diameter, hangs inside the chamber through a hole in the upper center of the chamber.

The study mouse was placed in the chamber and allowed to explore the chamber for 45 seconds. Conditioned stimulus (CS) i.e. buzzer signal was turned on and unconditioned stimulus (US) i.e. electric shock delivered through grid floor for 45 Seconds.

Animal learned to associate the buzzer with the impending foot shock and was capable of avoiding the foot shock by climbing the pole after buzzer signal. Avoidance response will be defined as climbing reaction time 10 Seconds.

Every mouse was subjected to maximum 5 trials on 1st day, and 24 hrs later, mouse was subjected to relearning trials (2nd day 3 trials and on 3rd day one trial) and transfer latency was noted to check the retention of Conditioned Avoidance Response (CAR) and Escape response (ER). Animals were screened by using this model and those who demonstrated at least one escape response either on day one or two were included in the study. The values were observed accordingly.

3. INCREASING CURRENT ELECTROSHOCK SEIZURES(ICES) :

Electro convulsometer

The increasing current electroshock seizures (ICES) was proposed by kitano et al and modified by Marwah et al was used for evaluation of the anticonvulsant effect of the drugs which used in this study.[31] A current of 2 mA electroshock to each mice via ear electrodes was delivered as a single train of pulses (for 0.2 s) was given with linearly increasing intensity of 2 mA/2 s using an Electro-convulsometer.

The current at which the appearance of tonic hind limb extension (HLE) occurred was recorded as the seizure threshold current. When no tonic hind limb extension was observed by a current of 30 mA, electroshock was terminated.

Findings was noted & validated. The mouse was allowed for free access to a standard pellet diet and tap water and further studies continued.

CHRONIC STUDY:

This study starts on the 2nd day after the acute study and drugs Phenytoin, Piracetam and Celastrus paniculatus was given daily in the dose which is mentioned above and was allowed for free access to a standard pellet diet and tap water, if any restriction of diet is needed will be followed.

The drugs were given till 22nd day, for a period of 21days and the methods (i.e) radial arm maze test, Pole climbing apparatus test is performed. Then observations are noted and validated.

After validation the mices was sacrified by using 80% carbondioxide by using euthanasia [32] chamber and Blood samples obtained through cardiac puncture for liver function tests and renal function tests and finally decapitation was done and whole brain was carefully removed from the skull. Liver and Kidney samples were sent to Department of Pathology, DSMCH for Histopathological examination.

The fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9% w/v sodium chloride solution and centrifuged & supernatant liquid was used for AChE, Nor-adrenaline, Dopamine ,Serotonin activity.

BIO-CHEMICAL TEST:

Validation of blood and Estimation of neurotransmitters were done by using UV-spectrophotometer, Fluorescence spectro-flurimeter from St. Joseph college of Arts & Science after getting the permission of the committee members, Tiruchirapalli. The values were Noted and evaluated. Blood investigations were performed at Friends diagnostics laboratory, Sastri road, Tiruchirapalli, Tamilnadu.

Estimation of Brain Acetyl‑cholinesterase Activity:

The method proposed by Hestrin was used for the estimation of whole brain acetyl-cholinesterase (AChE) activity and was based on the development of yellow color resulting due to the reaction of thiocholine with dithiobisnitrobenzoate ions.

Spectrophotometric measurements were made forth extent of rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase. The treatment of sample was first done with 5,5'‑dithionitrobenzoic acid followed by determination of optical density (at 412 nm) of the yellow color compound formed during the reaction every minute for a period of 2 min was measured. AChE activity was calculated using the following formula.

R = σ O.D × Volume of assay (3 ml)/E × mg of protein,

Where **R** = Rate of enzyme activity in "n" mole of acetylthiocholine iodide hydrolyzed/minute/mg protein,

σ O.D = Change in absorbance/minutes.

E = Extinction coefficient = 13600/M/cm statistical analysis.[33]

ESTIMATION OF NORADRENALINE AND DOPAMINE:

Reagents

1. 0.4M HCl

2. Sodium acetate buffer (pH 6.9)

3. 5M NaOH

4. 0.1 M Iodine solution (in Ethanol)

5. Na2 SO3 sol. ((0.5 g Na SO in 2 ml H O + 18 ml 5 M NaOH)

6. 10M Acetic acid

Procedure

To the 0.2 ml of aqueous phase, 0.05 ml 0.4 M HCl and 0.1 ml of EDTA / Sodium acetate buffer (H6.9)was added, followed by 0.1 ml iodine solution(0.1 M in ethanol) for oxidation.

The reaction was stopped after 2 min by addition of 0.1 ml Na_2SO_3 solution, 0.1 ml Acetic acid is added after 1.5 min.

The solution was then heated to 100°C for 6 min when the sample again reached room temperature, excitation and emission spectra was read from the spectrofluorimeter. The readings was taken at 330 -375nm for dopamine and 395 -485 nm for nor-adrenaline.[33]

ESTIMATION OF SEROTONIN

The serotonin content was estimated by the method of Schlumpf.

Reagents

1. Ophthaldialdehyde (OPT) reagent: (20 mg in 100 ml conc. HCl).

Procedure

To 0.2 ml aqueous extract 0.25 ml of OPT reagent was added. The fluorophore was developed by heating to 100°C for 10 min.

After the samples reached equilibrium with the ambient temperature, readings was taken at 360nm -470 nm in the spectrofluorimeter.[33]

STATISTICAL ANALYSIS

The expression of data was done as mean ± standard deviation. The normally distributed data was subjected to one‑way ANOVA followed by Dunnett's test. $P < 0.05$ was considered significant.

RESULTS:

Acute studies:

Acclimatization of the animals was done for 10 days and mice was grouped and training was given, followed by training for radial arm maze apparatus test was given and each animal was trained with 4 trials and with pole climbing apparatus each animal were given 9 trials and the drugs were given orally.

On Day 1,

- Group I (Control group) : Standard pellet diet and tap water.
- Group II, III and IV : Phenytoin suspension- 0.32mg, 0.48mg and 0.88mg respectively
- Group V, VI and VII : Piracetam- 5mg, 10mg and 20 mg respectively
- Group VIII, IX, X : Pure Malkangani oil 4mg, 8mg and 16mg respectively
- Group XI : Phenytoin & Piracetam 0.48mg and 10mg respectively
- Group XII : Phenytoin &(Celastrus paniculatus) Pure Malkangani oil 0.48mg and 8mg respectively.

Chronic studies:

The same drugs & dosages were continued for a period of 21 days and on 22nd day behavioral and biochemical assessment was performed.

Euthanasia were done using Carbon dioxide Anesthesia in CO_2 chamber and brain was dissected and centrifuged and biochemical analysis of neurotransmitters was performed. Histopathological examination was done and laboratory tests for Liver function test & Renal function tests was carried out.

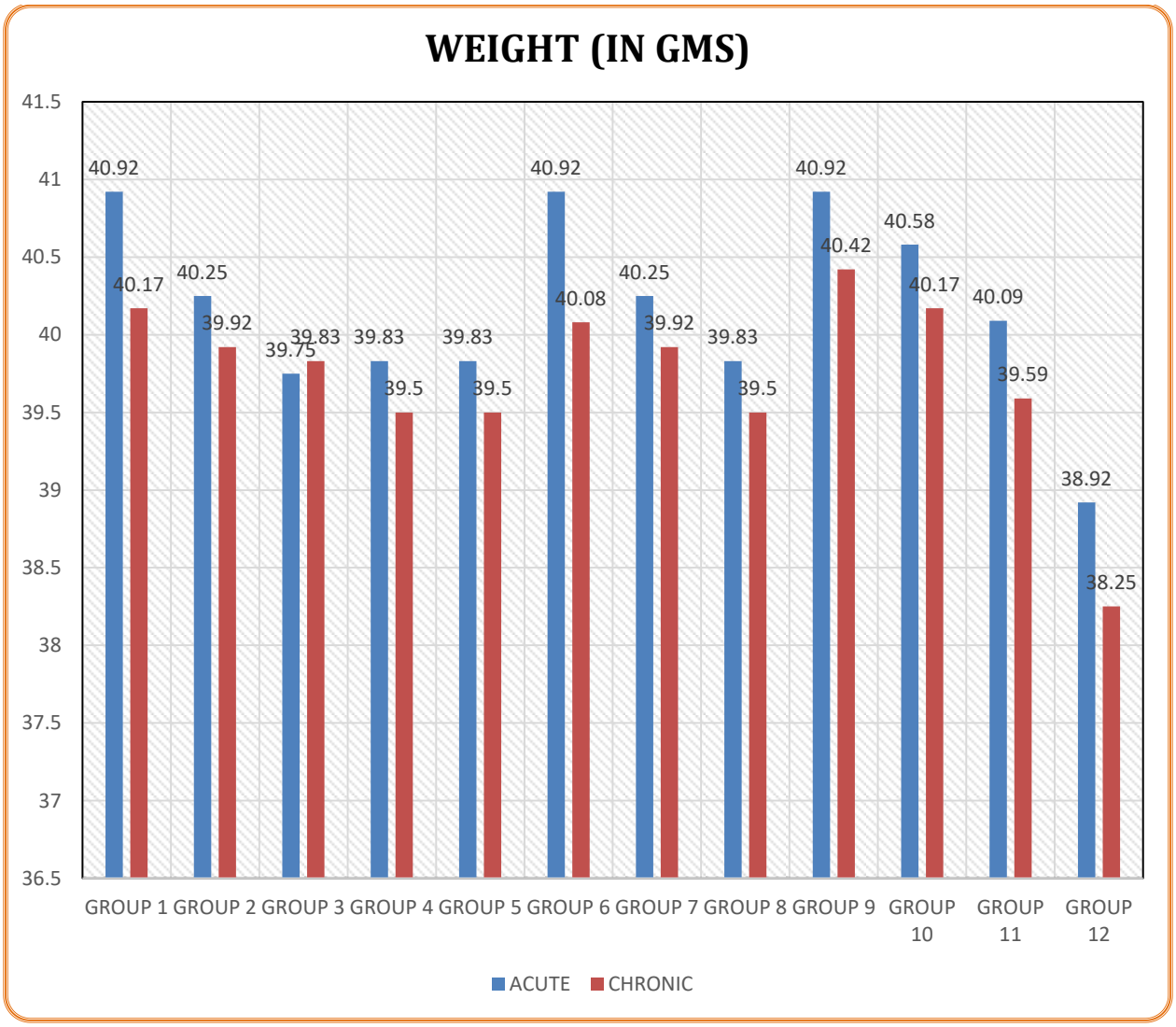
Behavioural study:

Group I: In both ACUTE & CHRONIC STUDY the weight is almost equal. Other parameters are not significant with p values ($P>0.05$).

A) **Radial arm maze test (RAM):** Right entries for acute & chronic study are 0.17, for wrong entry is 0.75, Re-entries is 0.41, Total entries are 0.45 and the time taken for that was 0.2 which is also not significant.

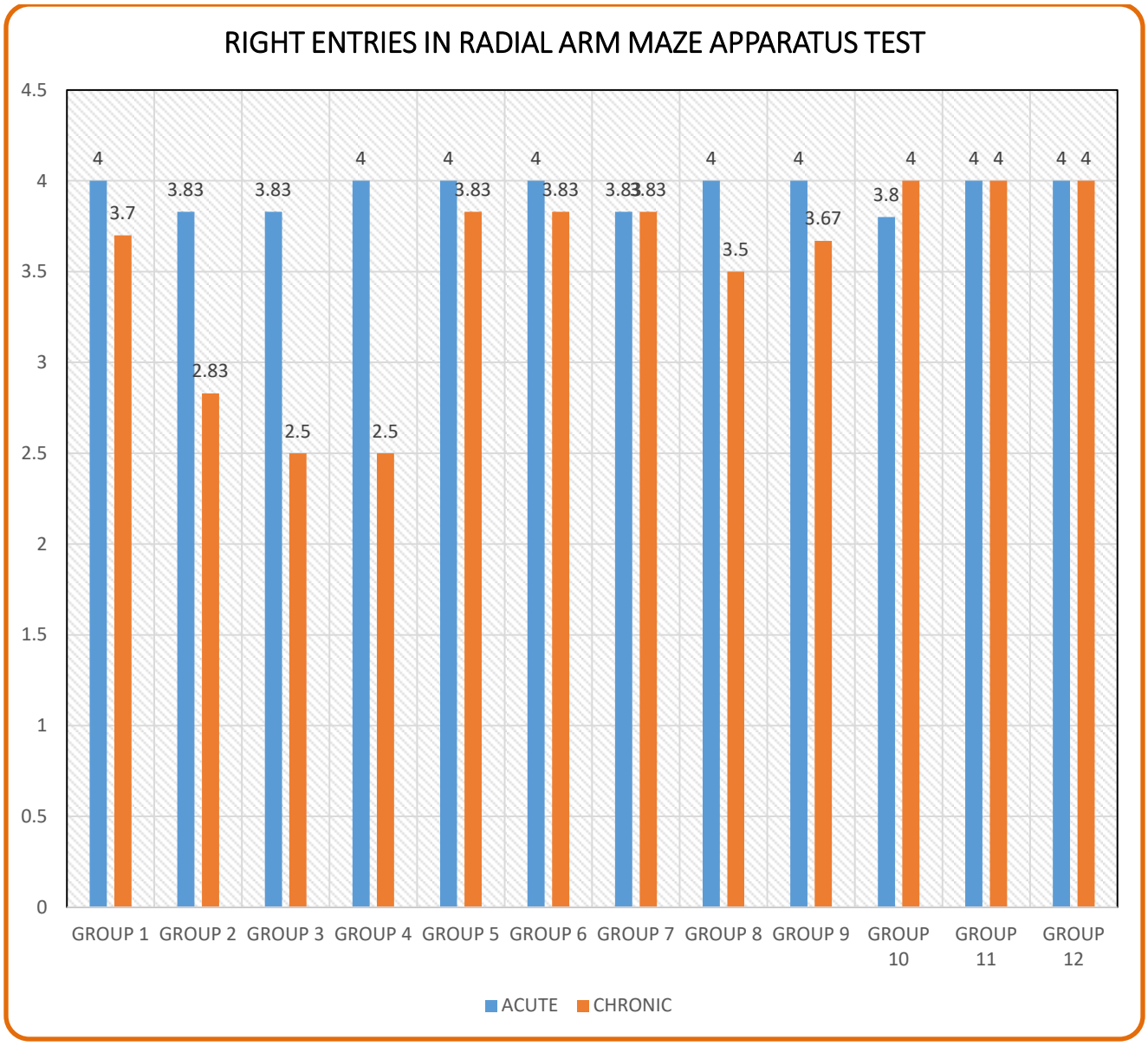
B) **Pole climbing apparatus test** values includes: Latency time: 0.08 and the Duration was 0.72, the Conditioned avoidance response /Escape response was: 3 ER and 3 CAR, 5 ER and 1 CAR.

C) **ICES** values were for only acute studies: 17.3333mv.



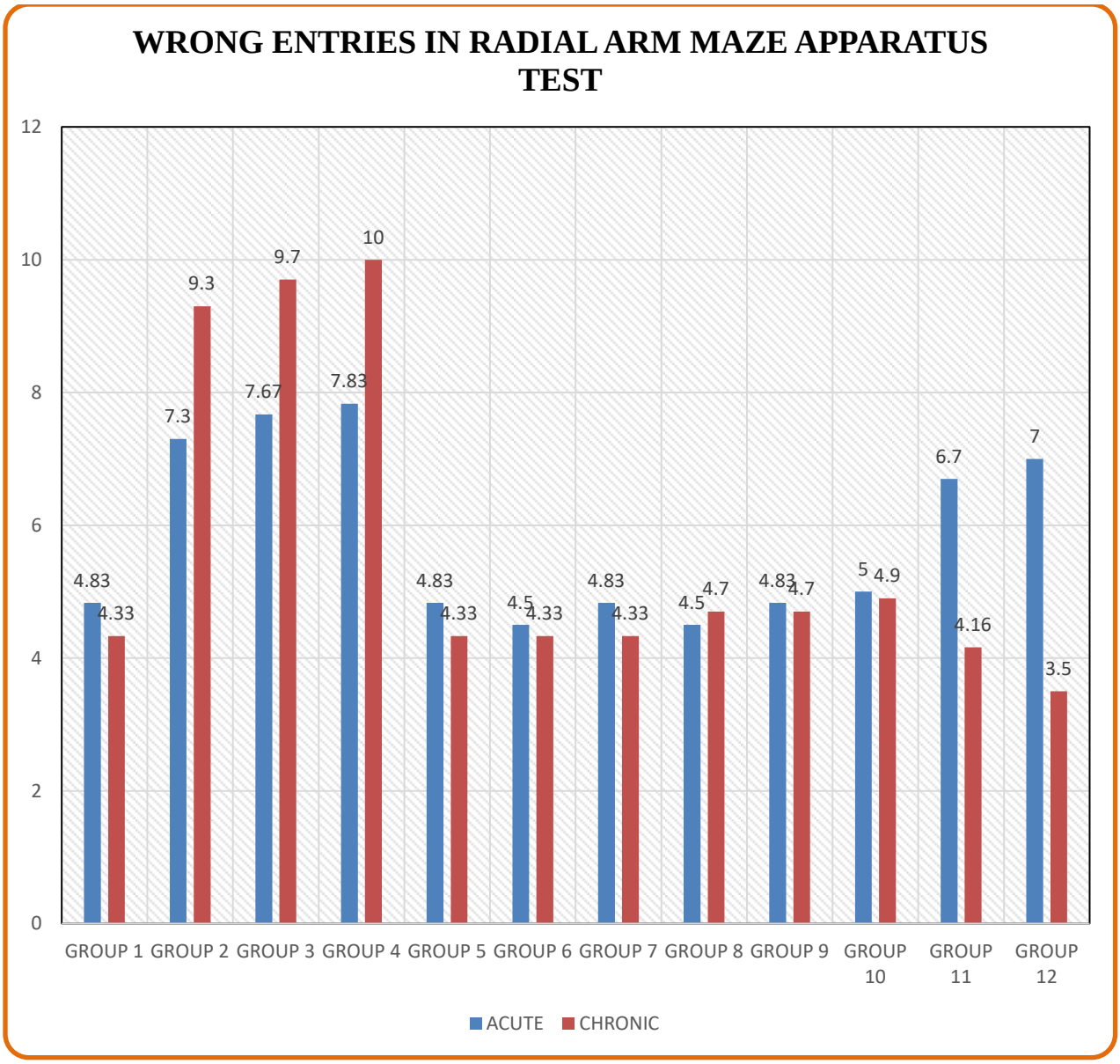
Comparison of weight in grams for Acute and Chronic studies.

In groups I, VI, IX the weight was almost equal in acute studies where as in both acute and chronic studies, it is reduced in group XII.



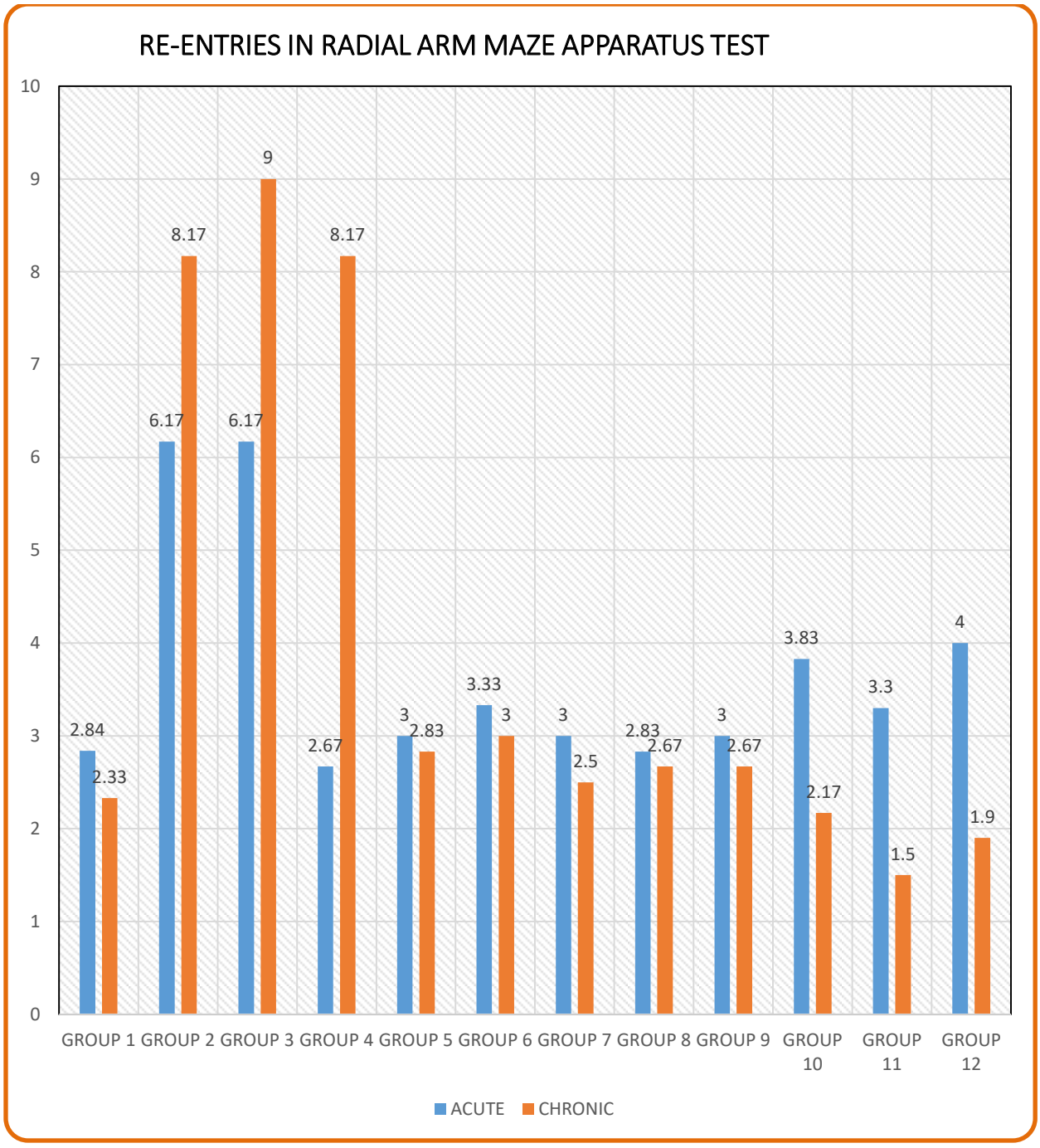
Comparison of Right Entries in Radial arm maze apparatus test for Acute and chronic studies.

In group II, III, IV chronic administration of phenytoin significantly reduced the right entries. In group XI & XII the right entries have improved compared to phenytoin group (group III)



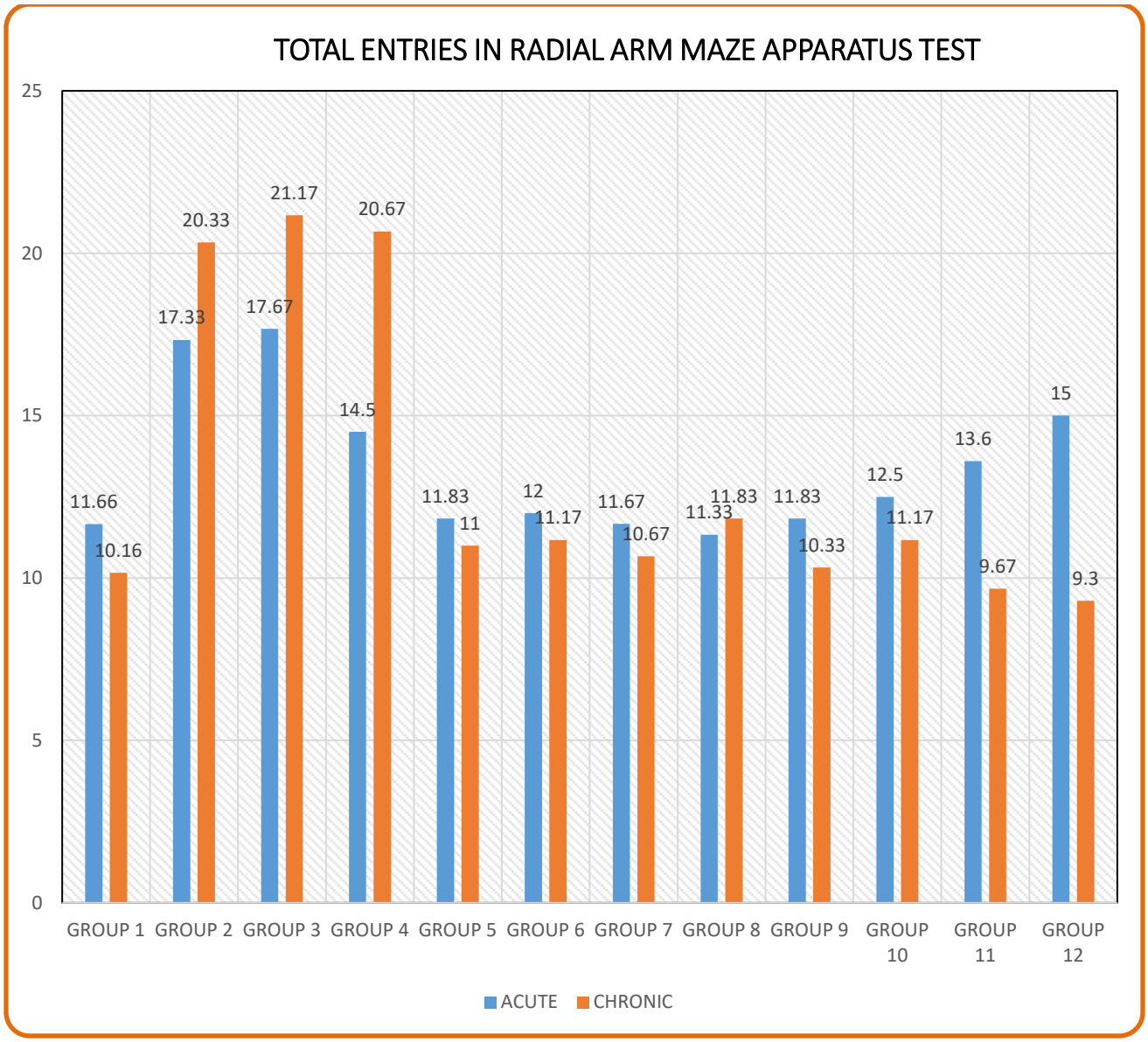
Comparison of Wrong Entries in Radial arm maze apparatus test for Acute and chronic studies.

In group II, III, IV chronic administration of phenytoin significantly increased the wrong entries. In group XI and XII there is a reduction in wrong entries compared to phenytoin group (group III)

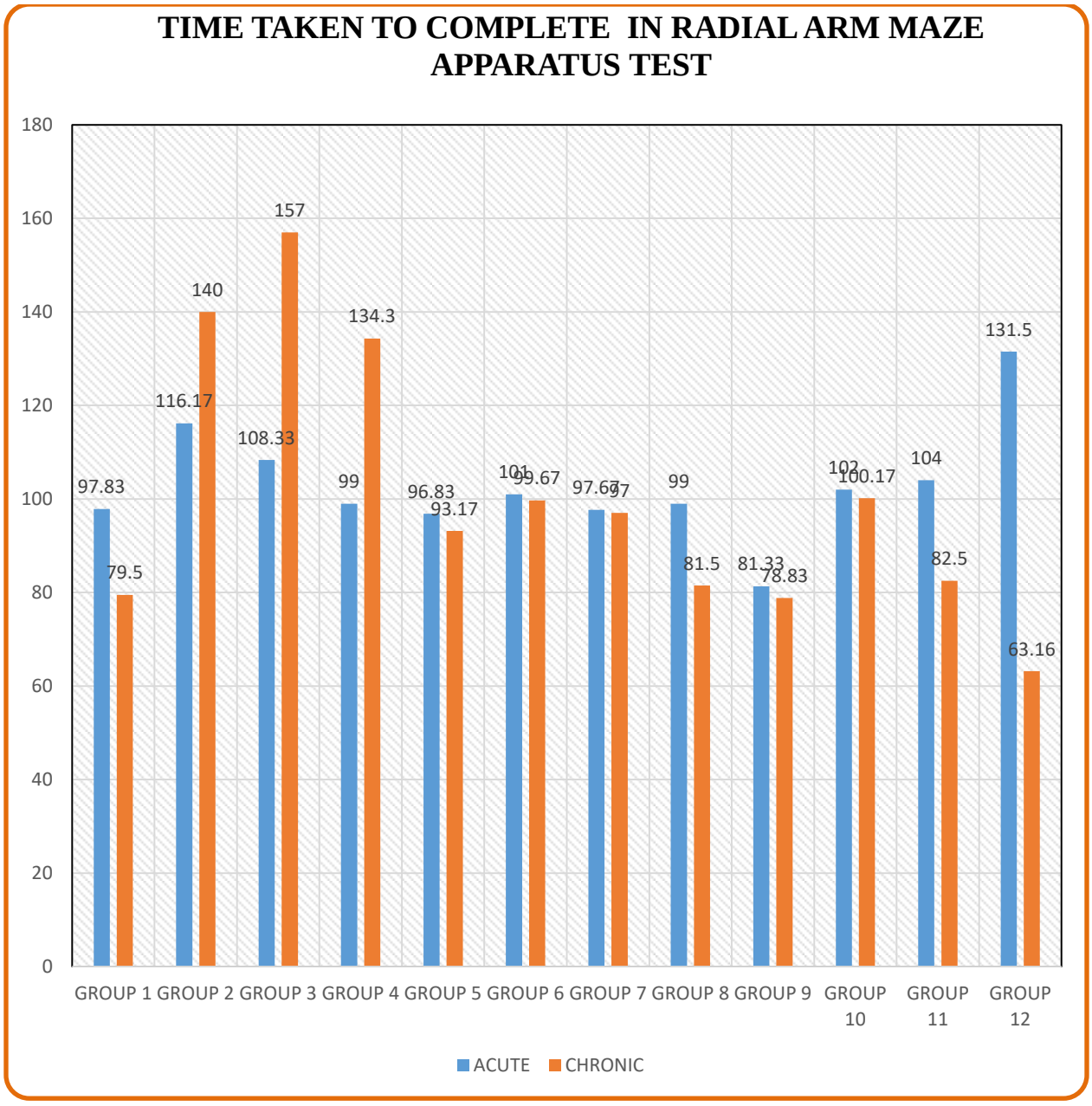


Comparison of Re-Entry in Radial arm maze apparatus test for Acute and chronic test.

In group II, III, IV chronic administration of phenytoin significantly Increased the Re-entries. In group XI & XII the re-entries are reduced compared to phenytoin group (group III)

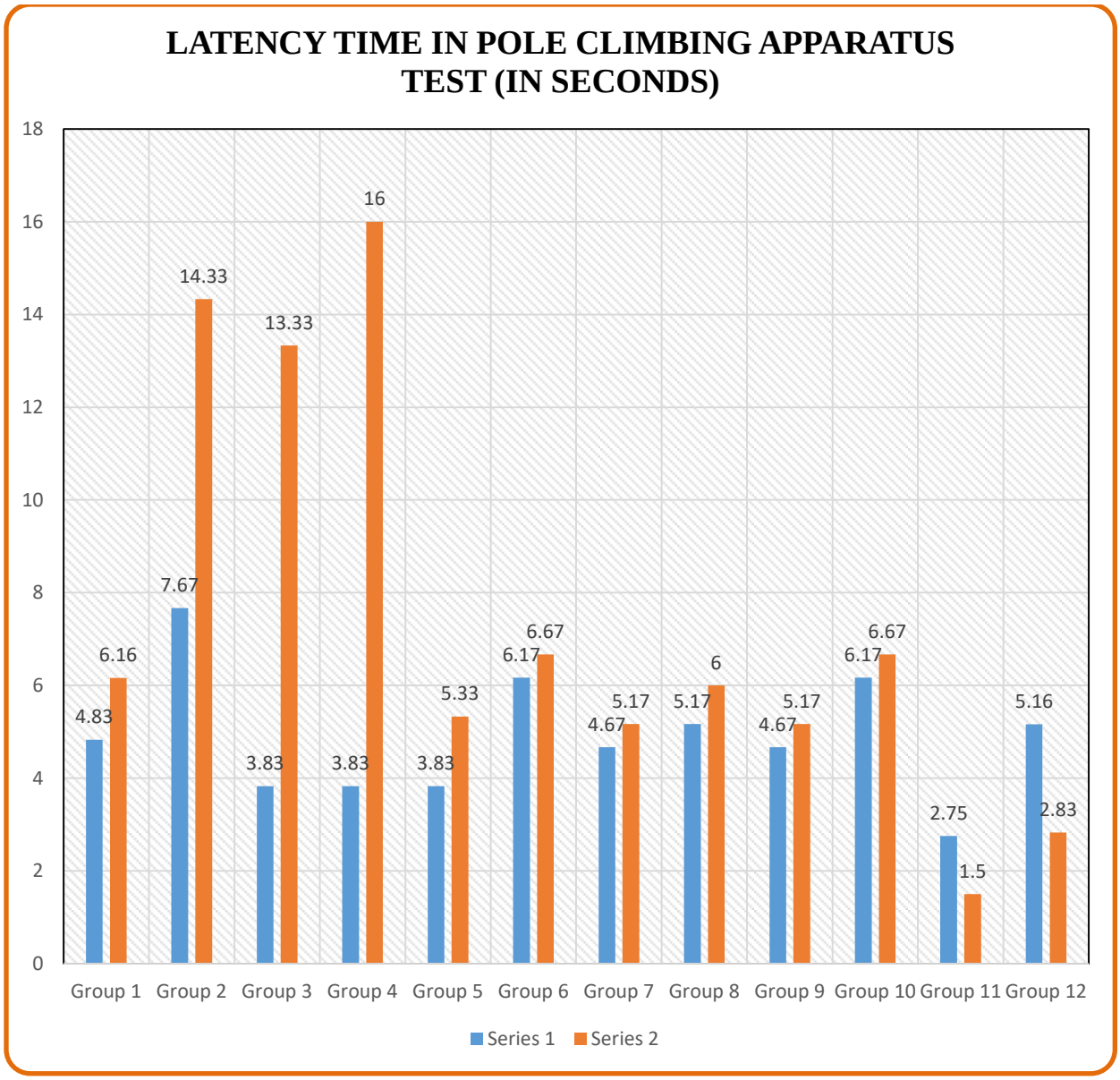


Results of Total Entries in Radial arm maze apparatus test for Acute and chronic studies. In group II, III, IV chronic administration of phenytoin significantly increases total entries. In group XI & XII there is a decrease in total entries compared to the phenytoin group (group III)



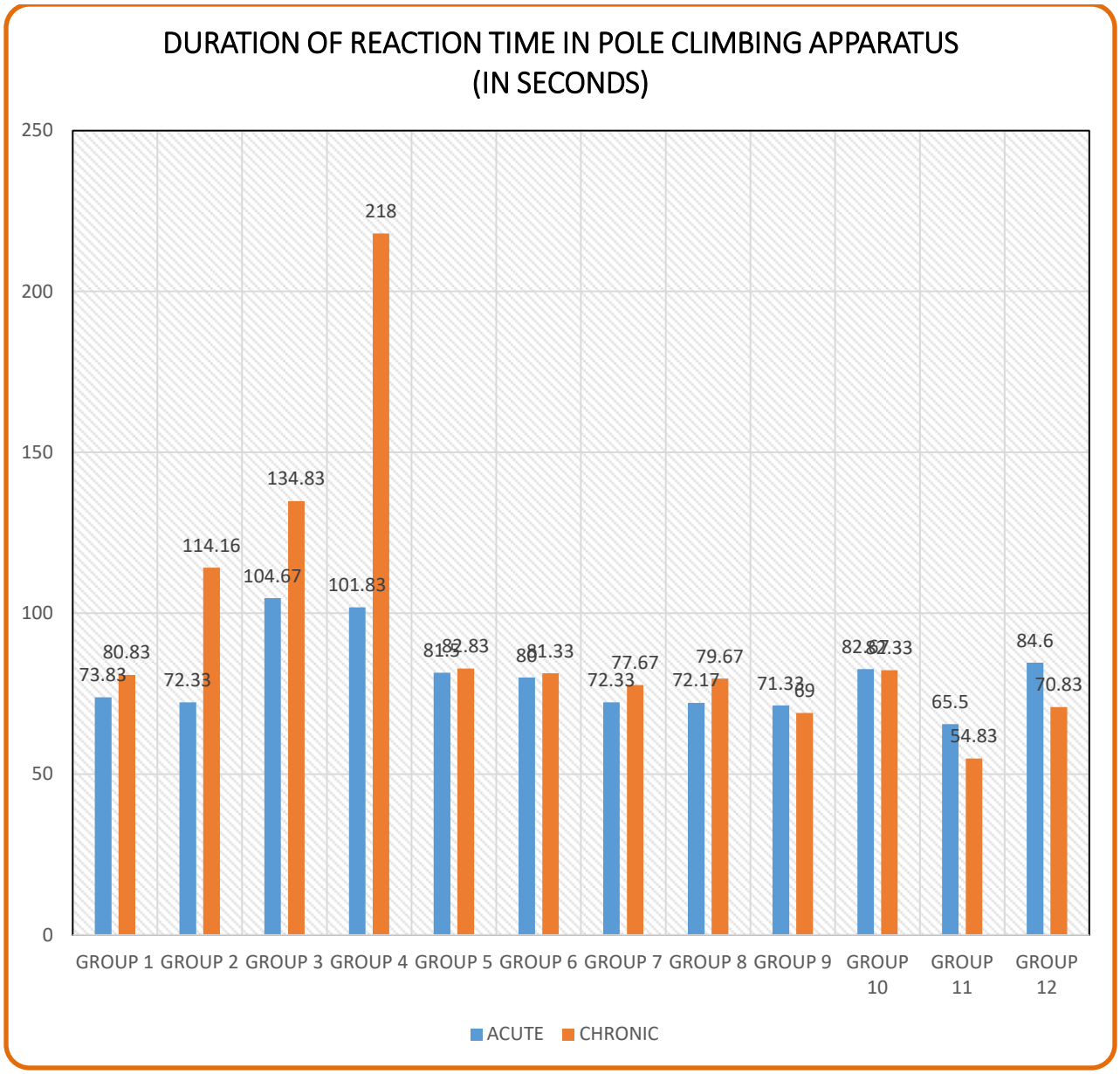
Comparison of Time taken (in seconds) in Radial arm maze apparatus test for Acute and Chronic studies.

In group II, III, IV chronic administration of phenytoin significantly increases the time taken to complete in radial arm maze. Time taken is decereased in group XI and XII compared to phenytoin group (group III).



Comparison of Latency time in pole climbing apparatus test for Acute and Chronic studies.

In group II, III, IV chronic administration of phenytoin significantly raised the latency time. Latency time is reduced in group XI and XII compared to phenytoin group (group III)

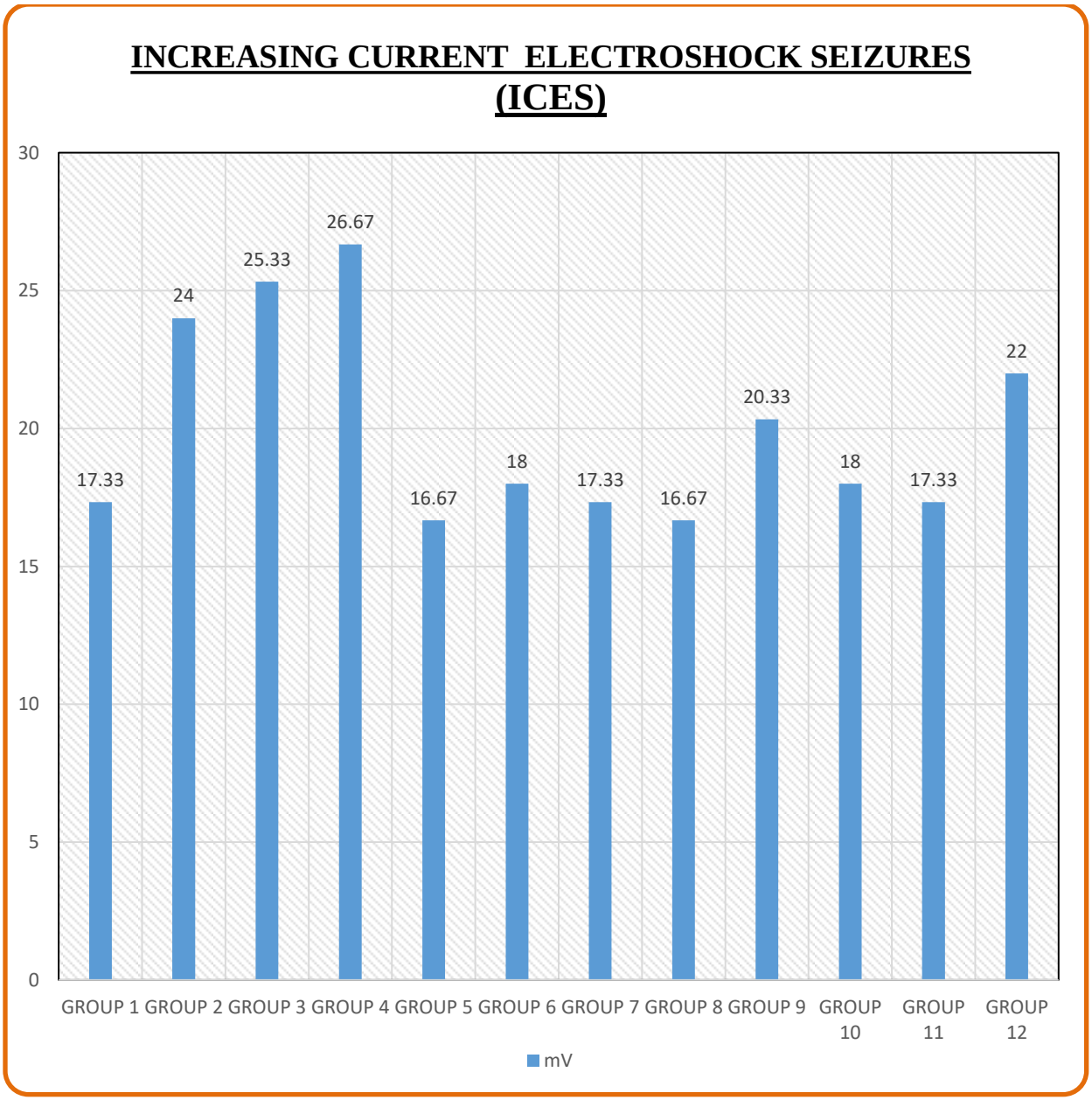


Results of duration of reaction time in pole climbing apparatus for Acute and chronic studies.In group II, III, IV chronic administration of phenytoin significantly raised the duration. The reaction time has significantly reduced in group XI and XII compared to phenytoin group (group III).

CONDITIONED AVOIDANCE RESPONSE (CAR) / ESCAPE RESPONSE (ER) IN POLE CLIMBING APPPARATUS:

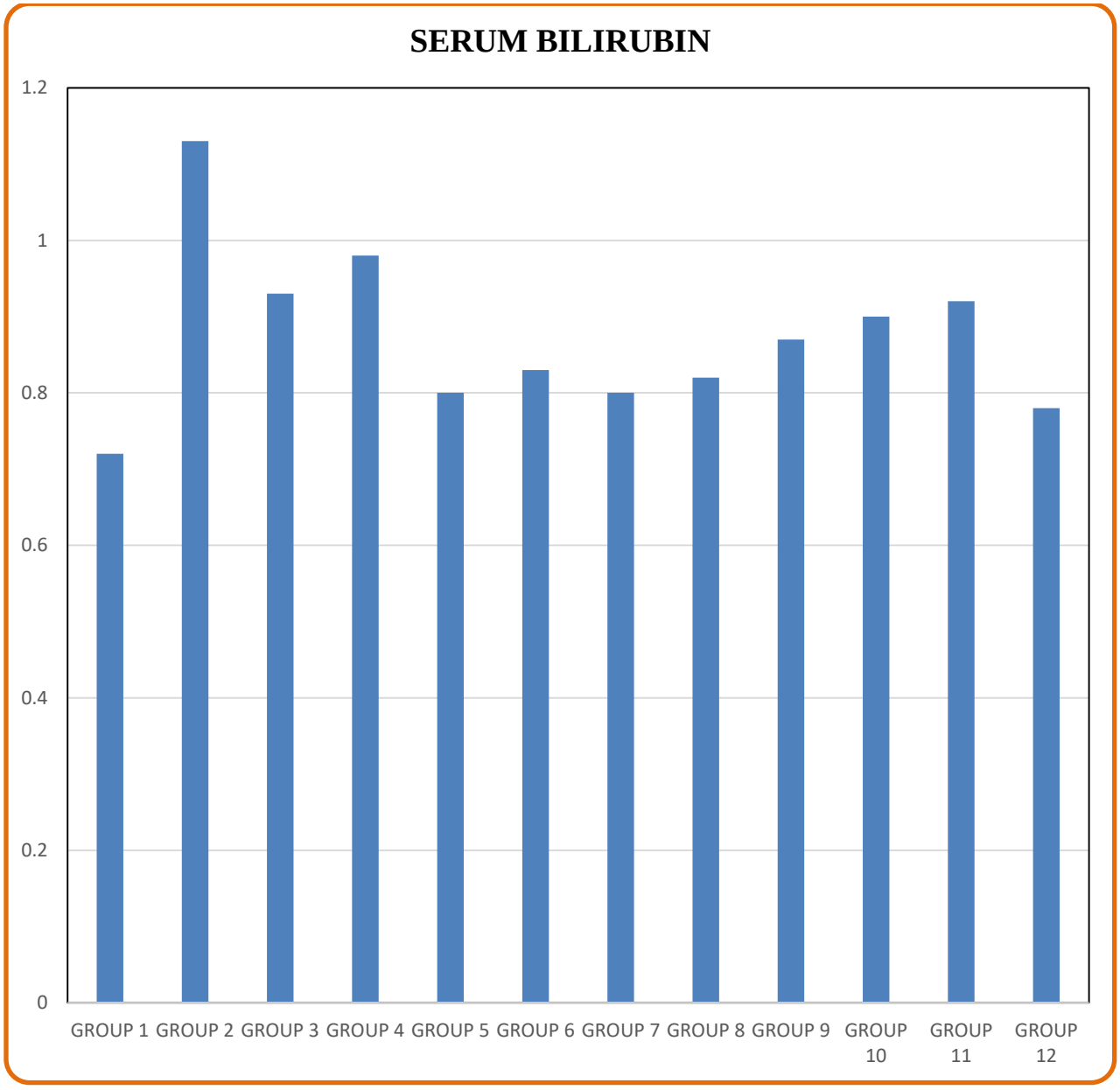
	Acute	Chronic
Group I	3- ER/3- CAR	3ER/3CAR
Group II	4ER/2CAR	6ER
Group III	4ER/2CAR	5ER/1CAR
Group IV	3ER/3CAR	5ER/1CAR
Group V	3ER/3CAR	3ER/3CAR
Group VI	3ER/3CAR	3ER/3CAR
Group VII	3ER/3CAR	4ER/2CAR
Group VIII	3ER/3CAR	4ER/2CAR
Group IX	2ER/4CAR	3ER/3CAR
Group X	3ER/3CAR	3ER/3CAR
Group X1	6ER	4ER/2CAR
Group XII	3ER/3CAR	3ER/2CAR

In group I, IX, X is almost equal (i.e) In group IX, X signifies result similar to the control group- 3ER/3 CAR. And in group XII is almost similar 3 ER/2CAR.



Effect on Increasing current electroshock seizures on Acute studies.

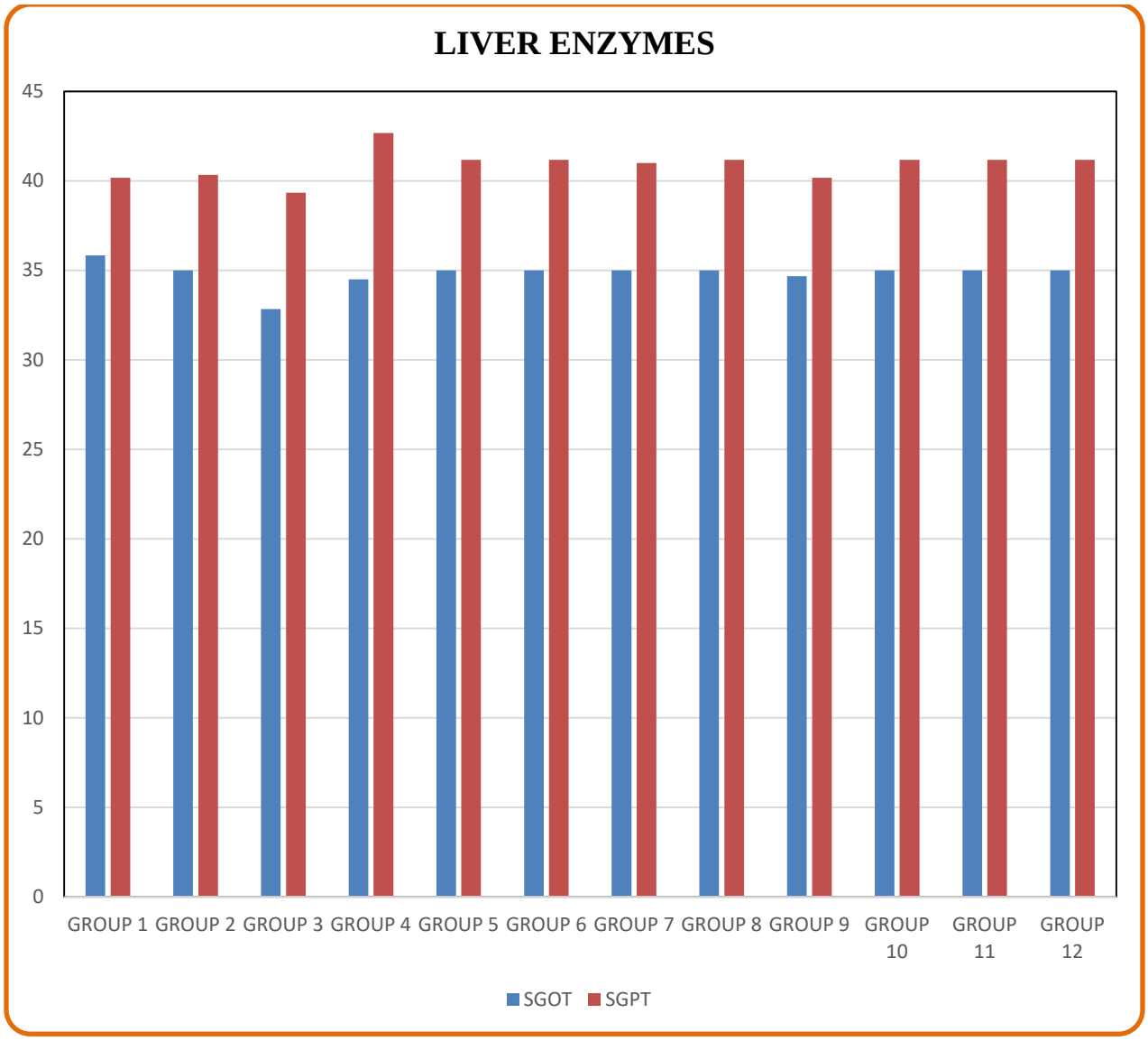
In group II, III, IV acute administration of phenytoin significantly increased the seizure threshold compared to other groups.



Results of Bilirubin levels:

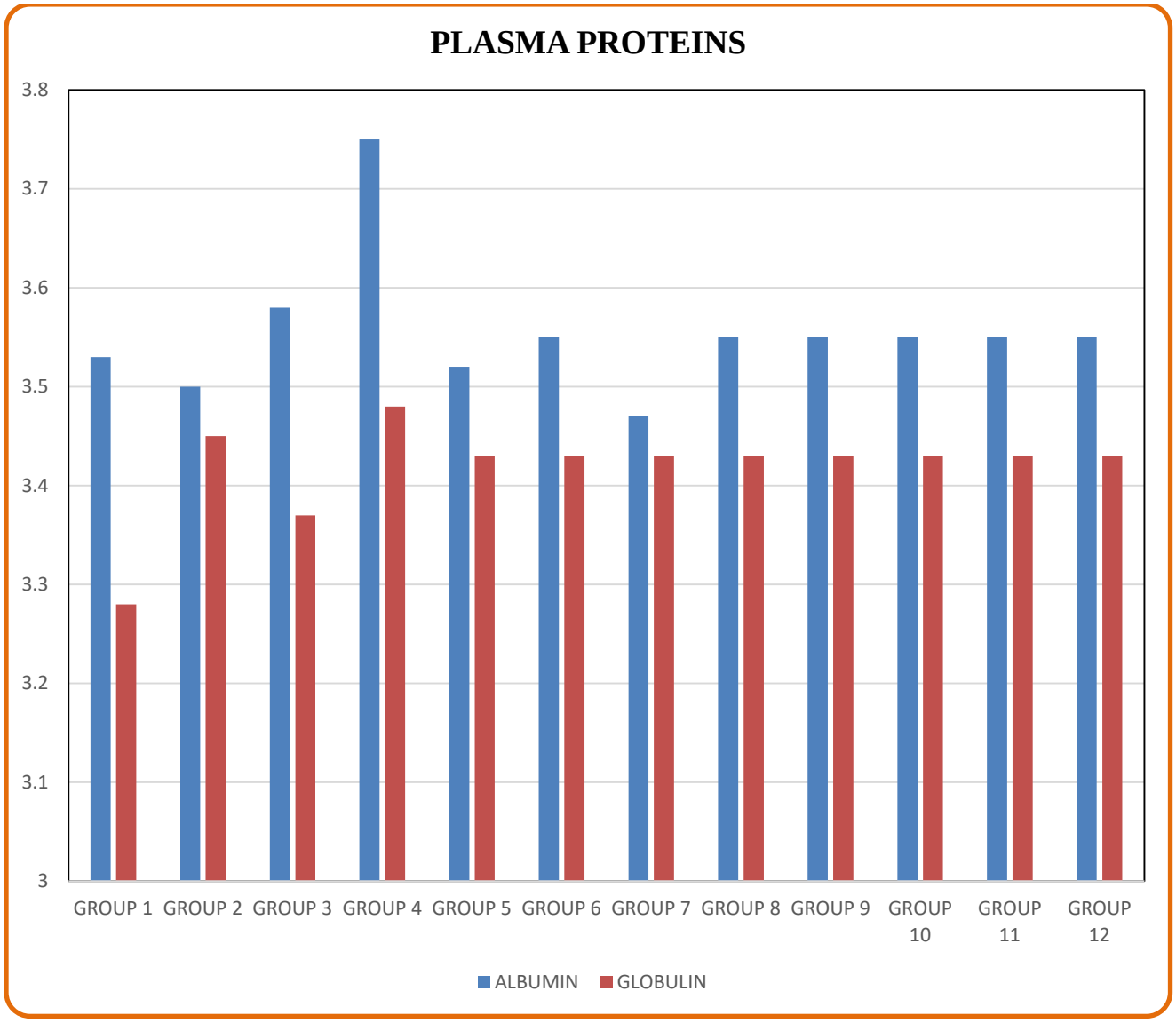
In group II, III, IV chronic administration of phenytoin significantly raised the levels.

Reduction in Bilirubin levels is better with group XII than group XI.



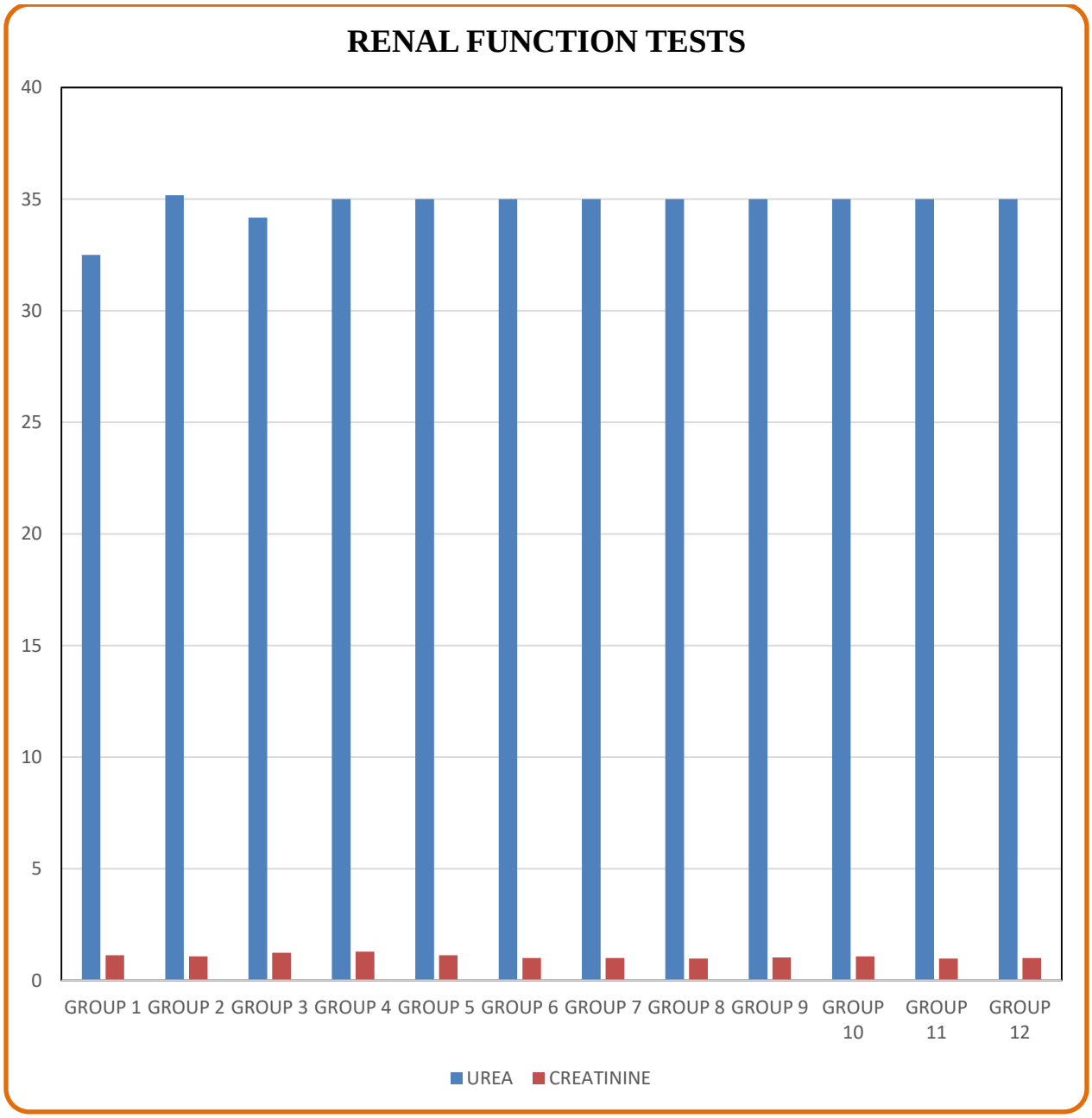
Comparison of Liver Enzymes levels, chronic administration of phenytoin causes raised levels.

No obvious changes in liver enzymes in all the groups.



Comparison of Plasma Proteins levels.

In groups II, III & IV chronic administration of phenytoin significantly reduced the plasma protein levels. The changes in plasma protein levels are comparable in all the groups.



Comparison of Renal Function tests levels: In all groups urea and creatinine are almost equal.

No significant changes in Urea and Creatinine are observed in all the groups.

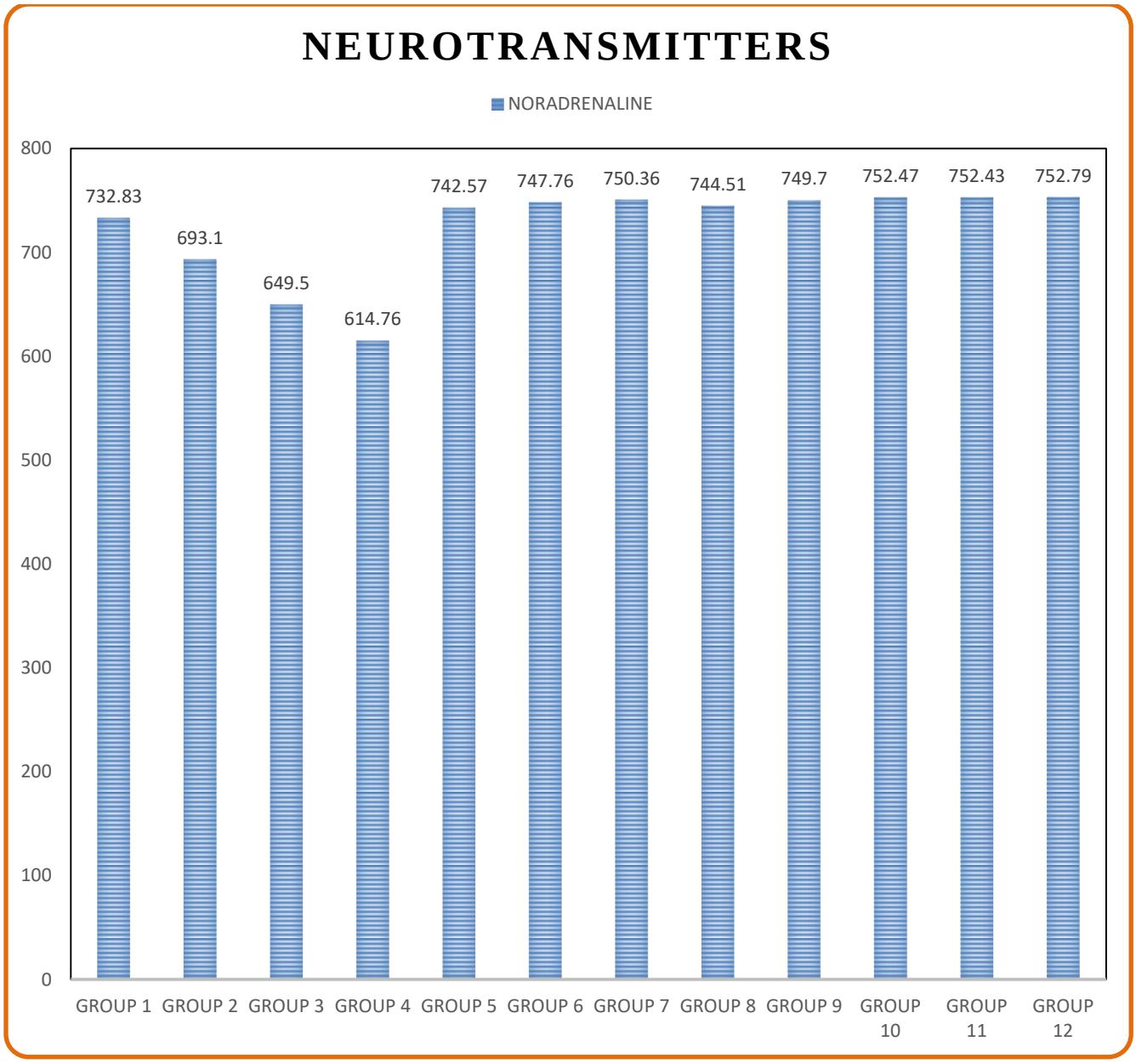
In **Group II** both ACUTE & CHRONIC STUDY the weight is almost equal which is not significant.(P>0.05) and in **Radial arm maze test (RAM):** Right entries for acute & chronic study is significant (P=0.04), for wrong entries in acute and chronic study is significant (P=0.01), Re-entries for acute and chronic study is also (P=0.03) significant.

Total entries for acute and chronic study is not significant (P=0.07)and the Time taken for both the study is significant(P=0.03) and in **Pole climbing apparatus test**: Latency time in acute and chronic study is significant (P=0.0007) and the Duration among both the study is also significant (P=0.003, in Conditioned avoidance response /Escape response were : 4 Escape response and 2 Conditioned avoidance response and in chronic study 6 Escape response were noted which denotes the cognition impairment, **ICES** values were in acute studies: (P=0.013) were also significant.

Group III, Acute and chronic the weight is almost equal which is not significant (P>0.05) and right entry is significant (P=0.01), wrong entry (P= 0.01), re-entry(P= 0.003) among total entries were (P=0.005)and the time taken were found to be (P= 0.04)and in pole climbing apparatus Latency time were (P=0.0003)and the duration were (P= 0.01), CAR/ER were 4ER/2CAR and in chronic study it is 6ER , In acute study ICES were P=0.001).

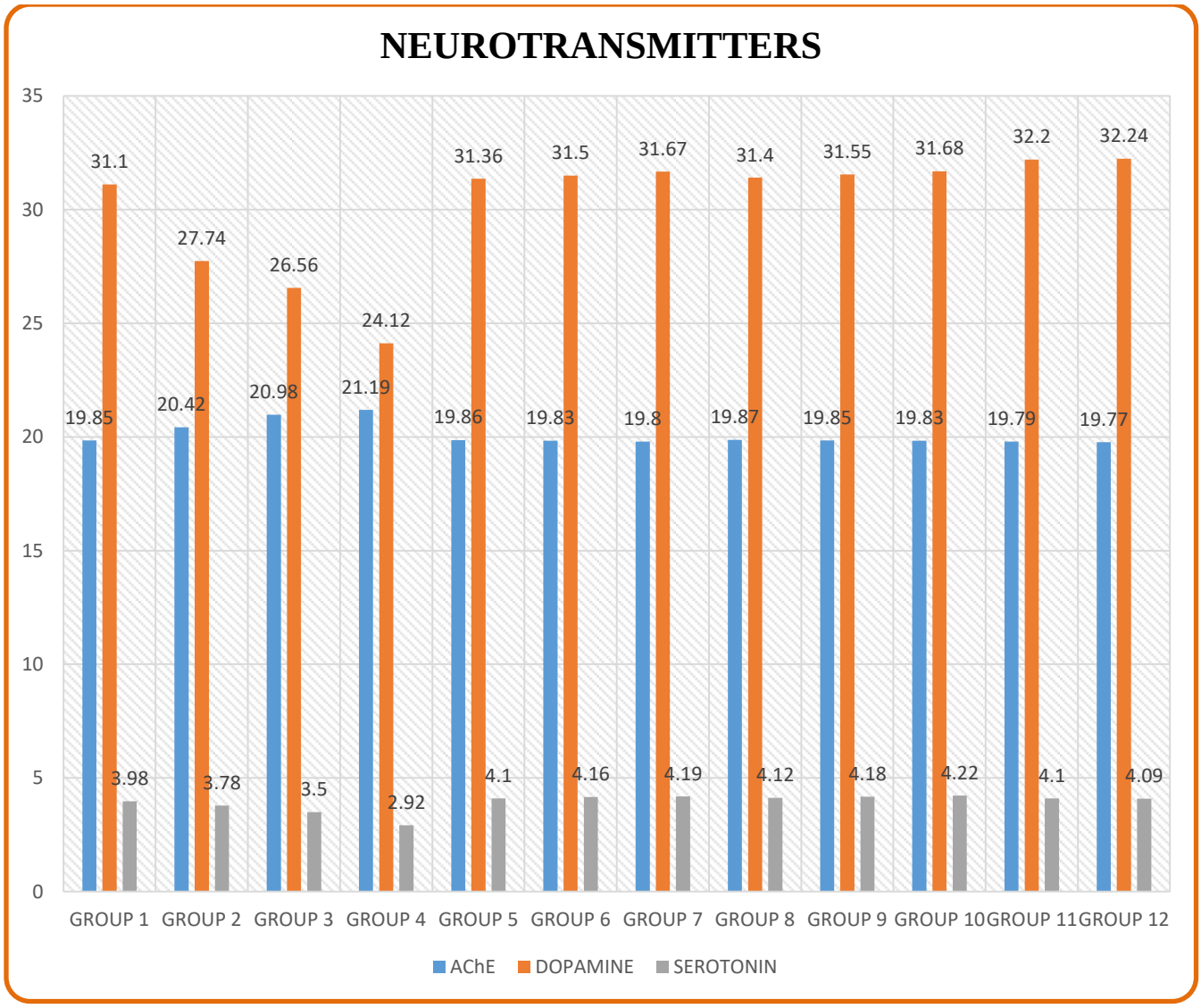
Group IV Acute and chronic the weight is almost equal which is not significant. (P>0.05) and right entry is (P=0.01) , wrong entries were (P= 0.002) , re-entry is (P= 0.03), total entry were (P= 0.028) and the time taken were (P= 0.007) , in pole climbing test latency time were (P= 0.004) , duration (P= .0001) and the CAR/ER were 4ER/2CAR and in chronic study 6ER , In ICES (P=0.0005).

Group V,VI,VII there was no significant difference in P values and in VIII, IX, X similar to previous groups and no significance is noted in P values, But in groups XI and XII there is no significance of P value noted but as such in acute and chronic comparison there is significance noted in the study.



Comparison of Neurotransmitter estimation between groups in Nor-Adrenaline after chronic studies.

In group II, III, IV chronic administration of phenytoin significantly reduced the neurotransmitter (Nor-adrenaline) levels. The changes in group XI and XII are comparable.



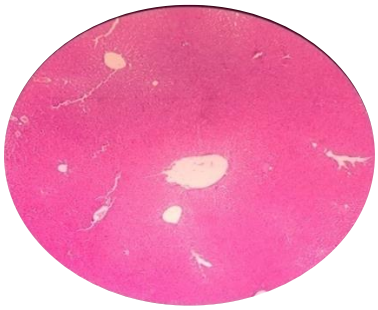
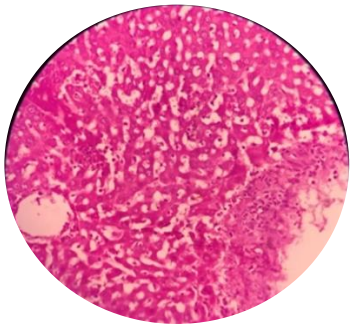
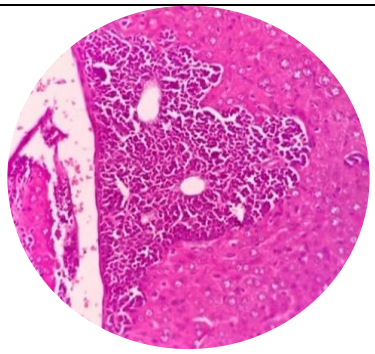
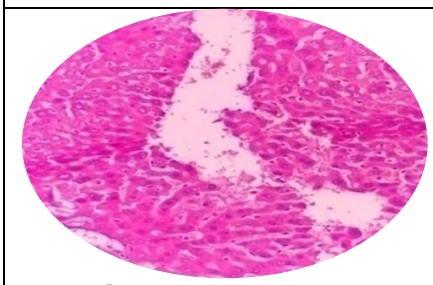
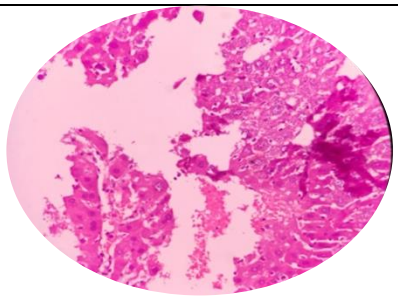
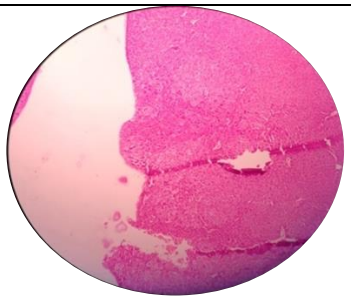
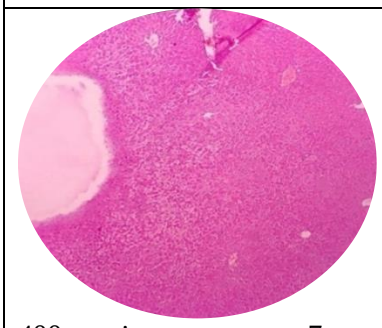
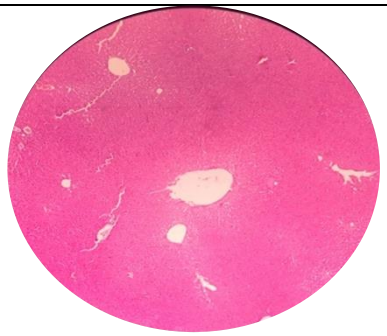
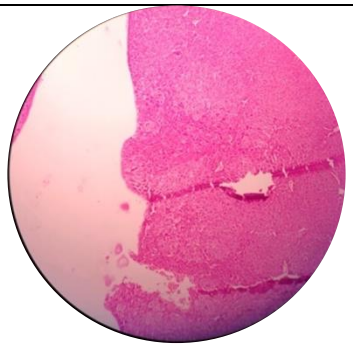
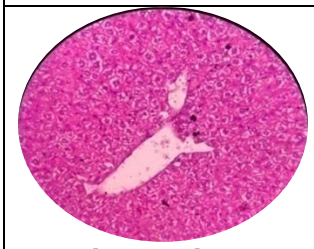
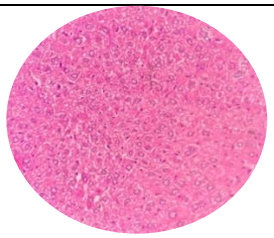
Comparison of Acetyl-cholineesterase, Dopamine, Serotonin after chronic studies.

In group II, III, IV chronic administration of phenytoin significantly decreased the dopamine and serotonin levels where as Acetyl-cholineesterase levels are slightly raised. In groups XI and XII the changes are comparable.

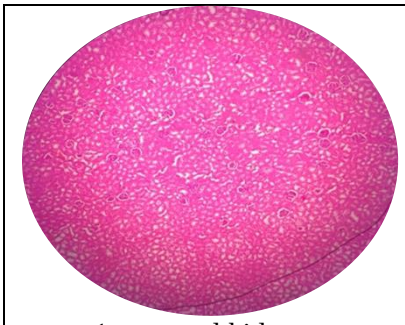
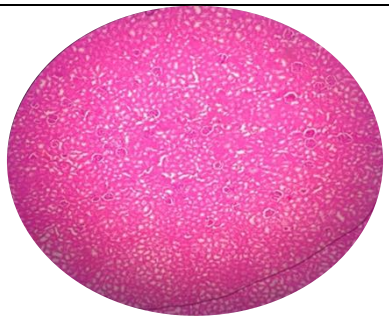
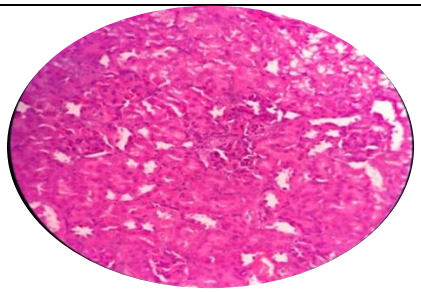
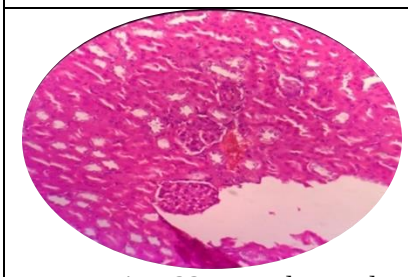
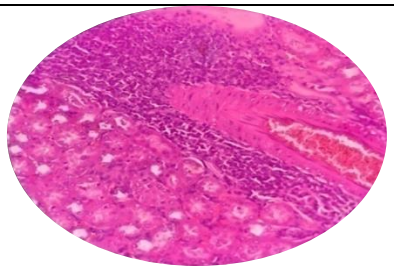
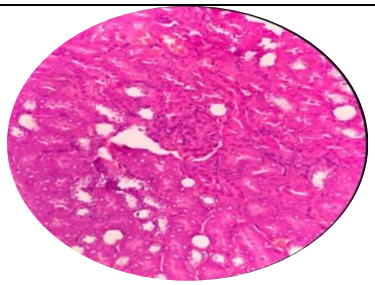
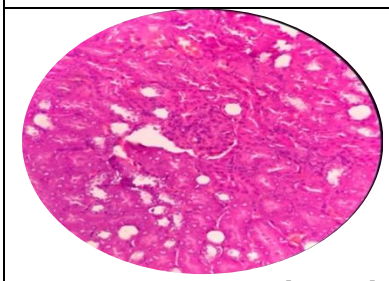
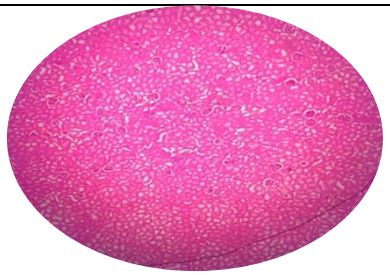
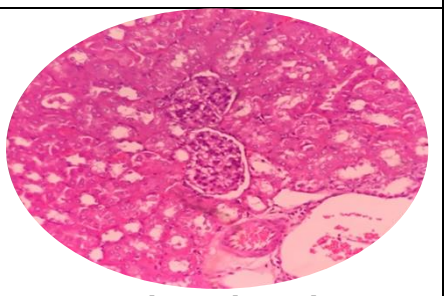
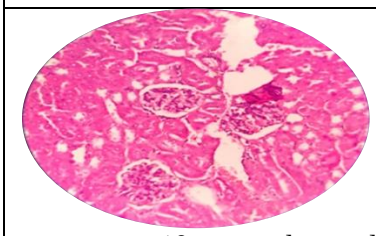
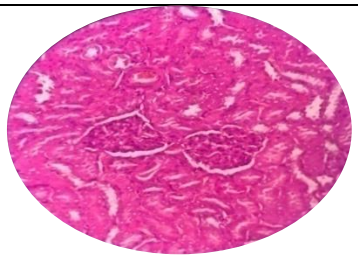
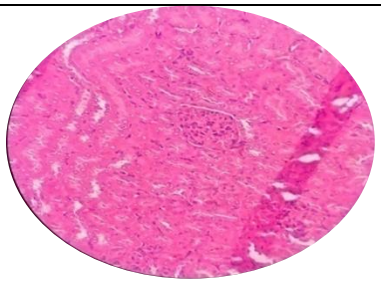
Liver function test was comparable in all the groups except Groups II, III and IV where we observed a little increase in bilirubin levels. But these differences were not significant ($p>0.05$). Liver enzymes, Plasma proteins and Renal function tests were comparable in all groups and the changes were found to be not significant ($p>0.05$).

Histopathological Examination using Eosin and Haematoxylin staining was done at Department of Pathology, Dhanalakshmi Srinivasan Medical College, Siruvachur, Perambalur and the findings were noted.

HISTOPATHOLOGICAL EXAMINATION OF LIVER:

 normal liver group 1	 8mg phenytoin with lymphocytes- group 2	 12mg phenytoin- group 3
 22mg phenytoin-group 4	 100mg piracetam-group 5	 kupfer cells- group 6
 400mg piracetam-group 7	 normal liver- group 8	 kupffer cells in plant extract-group 9
 normal portal tract with mild cytoplasmic vacuoles plant extract-group 10.	 inflammatory infiltrate of the portal tract- Group 11	 cytoplasmic vacuolization – group-12.

HISTOPATHOLOGICAL EXAMINATION OF KIDNEY:

 group 1 – normal kidney	 group 2 – normal kidney.	 group 3, 12mg phenytoin showing glomerular enlargement with destruction of tubules.
 group 4- 22mg glomerular enlargement with collapse of tubules.	 group 5- piracetam 100mg kidney perivascular lymphocytic infiltration.	 group 6- glomerular enlargement piracetam 400mg.
 group 7- glomerular enlargement piracetam 400mg.	 group 8 – normal kidney.	 group 9- glomerular enlargement seen in plant extract.
 group 10 -glomerular enlargement plant extract.	 group 11- glomerular enlargement prominent.	 group 12- glomerular enlargement

DISCUSSION:

Poor memory, lower retention and slow recall are common problems in today's stressful and competitive life. The herbal drugs have shown the promising effect in the treatment of memory loss. The herbal products acting on the brain are called as Nootropic herbs ("Nootropic" is derived from Greek and means acting on the mind) and their isolated constituents referred to as **smart drugs**. Memory enhancer herbs enhance the memory and increase blood circulation in the brain. The herbs act either by improving memory or preventing neuro-degeneration by their antioxidant and anti-inflammatory activities.

The present study was aimed to determine the usefulness of co-administration of Piracetam and *Celastrus paniculatus* with clinically used Anti-epileptic drugs in epileptic patients. Our study results revealed that Piracetam or *Celastrus paniculatus* when co-administered with Phenytoin impressively reverted the cognitive impairment produced by phenytoin. *Celastrus paniculatus* supported the results as obtained for established combination with piracetam. At the lower dose of 125 mg/kg, the enhancement in the percentage alternation was seen but did not show any significance, statistically. However, this dose was effective in reversal of Phenytoin-induced cognitive impairment.

The results showed that phenytoin with the dose of (12–22 mg/kg, oral) had an adverse effect on the cognitive function in chronic studies. Weak memory and impaired learning ability are the most common symptoms of cognitive impairment.

Pharmacotherapy with psychoactive drugs is available for these ailments however they are not effective in all cases and exerts numerous side effects especially upon long term administration. Series of paradigms for evaluation of memory performance is carried out on different mechanisms. Various mazes are used conventionally to assess the learning and memory in animal models. Eight Radial arm maze apparatus test performance is an appetite motivated task and is also useful to assess the spatial reference as well as spatial working memory performance and about the agents that affect these processes. Pole climbing apparatus is used to study cognitive function, mainly a response to conditioned stimuli during learning & its retention.

In our study, Phenytoin (12 mg/kg, p.o.) prominently increased the "brain AChE activity" while on the other hand Piracetam (200mg/kg) and *Celastrus paniculatus* (250 mg/kg, p.o.) lowered the "brain AChE activity" (availability of ach is more) i.e. affirming the countervailing action of these drugs on the cholinergic system. The impairment caused due to Phenytoin on learning and memory is due to interference in the cholinergic system also it lowers the brain Ach levels. It is worth noting that Phenytoin at 8 mg/kg, p.o., did not showed any impairment and increased AChE levels. This results is contrary to the results obtained from the study by Dubey s et al.[30] Piracetam belongs to pyrrolidones group most of which are known to influence cholinergic functions.

In this study, AChE activity was reduced by Piracetam as well as by Celastrus paniculatus in brain. It is important to know an interesting fact in this context that co-administration of Phenytoin with Piracetam and Celastrus paniculatus apparently increased the Phenytoin-induced sharp rise in total brain AChE level showing the countervailing action of the cholinergic system.

Thus, it can be said that Piracetam and *Celastrus paniculatus* when they were given as adjuvant therapy with Phenytoin, it reverted the adverse effect produced on the cholinergic system. It is necessary to explore the complete potential of *Celastrus paniculatus* in improving the Phenytoin-induced cognitive impairment and getting the right stand in the current anti-epileptic drug therapy.

In our study phenytoin on oral administration not only decreased Ach levels causing cognition impairment and also reduces nor-epinephrine levels, but the other neurotransmitters like dopamine and serotonin is also found to be affected. Piracetam on co-administration neutralize the NT levels caused by phenytoin and the test drug (Celastrus paniculatus) also causes increase in Dopamine, Nor-adrenaline and serotonin levels contrary to the study done by Bhanumathy e*t al* in which the contents of NE, DA and 5-HT and their metabolites in the brain were significantly decreased in drug treated group. [31]

In view of certain CNS disorders such as depression, psychosis, we sacrificed the animals and evaluated the NTs levels to estimate the exact magnitude of the problem.

In other studies *Celastrus paniculatus* oil on oral administration lead to lower concentration of Nor adrenaline, Dopamine and Serotonin which is discordant to our results.[34]

LIMITATION OF THE STUDY:

1. In our study we have examined whole brain for the neurotransmitters estimation. Concentrations of them in individual parts of the brain, not been determined which would be more informative.

2. Study was performed with commercially available oil preparation. Value of the study would have been higher if done using seed extract preparation.

3. In this study, the drugs have been given orally. If it would have been given intra-peritoneally it would have been more enlightening.

SUMMARY AND CONCLUSION:

In this study we have evaluated the cognition impairment induced by phenytoin with increasing dose that is 8mg/kg, 12mg/kg, 22mg/kg in animal models. But with this dose the cognition impairment with 8mg/kg was not produced with single dose of Phenytoin in acute study but with chronic administration of phenytoin the same dose produced cognition impairment.

Moreover, the standard drug piracetam did not produce significant effect in normal animals. But with cognition impairment, significantly improved cognition. Even with *Celastrus paniculatus* oil it signifies the same effect (i.e) when there is cognition impairment it reverted the impairment produced by phenytoin.

Celastrus paniculatus has long been used in ayurvedic medicine for its potent medicinal properties. In our study C. paniculatus oil was observed to have remarkable effects in raising the levels of nor epinephrine (NE), dopamine (DA) and serotonin (5-HT) in the brain. Significant improvement was also observed in the retention ability of the drug treated mice. So it has been established that improvement in cognition is noted when there is impairment.

There is no significant difference in improving the cognition impairment between Celastrus paniculatus group and piracetam group. Further studies are needed to evaluate its efficacy, safety and more on this aspect in the form of clinical trials and animal studies to determine cognition improvement in patients taking anti-epileptic therapy especially phenytoin for seizure disorder and will ensure full anti-epileptic effect without causing cognition abnormalities.

- housed in polypropylene cages with false mesh to avoid coprophagy
- standard pellet diet
- TAP WATER
- controlled temperature (25 ± 2°C)
- humidity (50-55%) and light (12h-light-dark cycle)
- Baseline weight measured

ALGORITHM

Animals are grouped as follows

GROUP I
normal control

GROUP II,III,IV
Phenytoin
8,12,22mg/kg

GROUP V,VI,VII
Piracetam
125,250,500mg

GROUP VIII,IX,X
Celastrus paniculatus
100mg,200mg,400mg

GROUP XI
Phenytoin 12mg/kg
Piracetam 250mg/kg

GROUP XII
Phenytoin 12mg/kg
celastrus paniculatus 200mg/kg

Animals are trained accordingly

- Three independent assessor will be training each animal for 5 miniutes for 3days with intervelled trials.
- The drugs are administered post 3rd day and the observations are made for acute study
- Followed by chronic studyon 22nd day..

Drugs are administered accordingly

Drugs administered between 10 am and 12 am

(phenytoin prior 2 hours)

(piracetam and celastrus paniculatus prior 1 hour)

Prior observation

Pole climbing apparatus

Radial arm maze apparatus

ICES

Acute study (within 24 hrs)

Results obtained

On 21th day 2 hrs post administration of last dose

Pole climbing apparatus

Radial arm maze apparatus

Chronic study (on 22th day)

Animals are sacrificed by 80% carbondioxideanaesthesia using euthanasia

Blood samples obtained through cardiac puncture for LFT & RFT

Whole brain specimen is homogenized with tissue homogenizer.

Estimation of Acetylcholinesterase

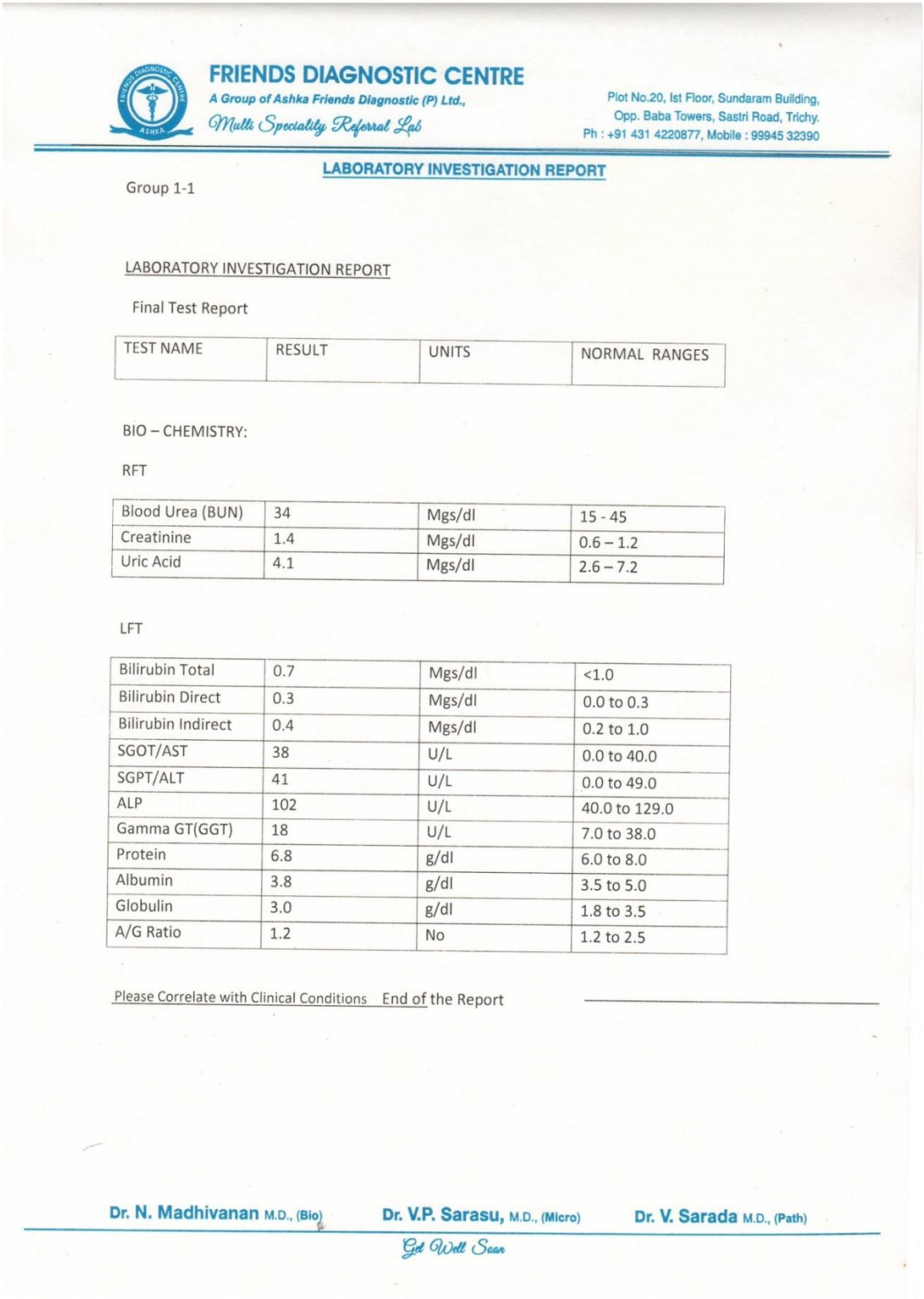
Estimation of dopamine and noradrenaline

Estimation of serotonin

Results obtained

ANNEXURE-I

LABORATORY INVESTIGATIONS



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LABORATORY INVESTIGATION REPORT

Group 1-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	34	Mgs/dl	15 - 45
Creatinine	1.4	Mgs/dl	0.6 – 1.2
Uric Acid	4.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	0.7	Mgs/dl	<1.0
Bilirubin Direct	0.3	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.4	Mgs/dl	0.2 to 1.0
SGOT/AST	38	U/L	0.0 to 40.0
SGPT/ALT	41	U/L	0.0 to 49.0
ALP	102	U/L	40.0 to 129.0
Gamma GT(GGT)	18	U/L	7.0 to 38.0
Protein	6.8	g/dl	6.0 to 8.0
Albumin	3.8	g/dl	3.5 to 5.0
Globulin	3.0	g/dl	1.8 to 3.5
A/G Ratio	1.2	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

Dr. N. Madhivanan M.D., (Bio) **Dr. V.P. Sarasu,** M.D., (Micro) **Dr. V. Sarada** M.D., (Path)

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LABORATORY INVESTIGATION REPORT

Group 2-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	33	Mgs/dl	15 - 45
Creatinine	0.5	Mgs/dl	0.6 – 1.2
Uric Acid	5.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	1.1	Mgs/dl	<1.0
Bilirubin Direct	0.5	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.6	Mgs/dl	0.2 to 1.0
SGOT/AST	35	U/L	0.0 to 40.0
SGPT/ALT	39	U/L	0.0 to 49.0
ALP	100	U/L	40.0 to 129.0
Gamma GT(GGT)	15	U/L	7.0 to 38.0
Protein	6.3	g/dl	6.0 to 8.0
Albumin	3.4	g/dl	3.5 to 5.0
Globulin	3.1	g/dl	1.8 to 3.5
A/G Ratio	1.8	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

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LABORATORY INVESTIGATION REPORT

Group 3-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	36	Mgs/dl	15 - 45
Creatinine	1.1	Mgs/dl	0.6 – 1.2
Uric Acid	3.9	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	1.0	Mgs/dl	<1.0
Bilirubin Direct	0.2	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.8	Mgs/dl	0.2 to 1.0
SGOT/AST	33	U/L	0.0 to 40.0
SGPT/ALT	39	U/L	0.0 to 49.0
ALP	99	U/L	40.0 to 129.0
Gamma GT(GGT)	33	U/L	7.0 to 38.0
Protein	6.2	g/dl	6.0 to 8.0
Albumin	3.1	g/dl	3.5 to 5.0
Globulin	3.5	g/dl	1.8 to 3.5
A/G Ratio	1.8	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

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LABORATORY INVESTIGATION REPORT

Group 4-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	37	Mgs/dl	15 - 45
Creatinine	0.6	Mgs/dl	0.6 – 1.2
Uric Acid	5.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	0.7	Mgs/dl	<1.0
Bilirubin Direct	0.1	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.6	Mgs/dl	0.2 to 1.0
SGOT/AST	31	U/L	0.0 to 40.0
SGPT/ALT	43	U/L	0.0 to 49.0
ALP	125	U/L	40.0 to 129.0
Gamma GT(GGT)	19	U/L	7.0 to 38.0
Protein	6.9	g/dl	6.0 to 8.0
Albumin	3.8	g/dl	3.5 to 5.0
Globulin	3.4	g/dl	1.8 to 3.5
A/G Ratio	2.1	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

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LABORATORY INVESTIGATION REPORT

Group 1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	37	Mgs/dl	15 - 45
Creatinine	0.6	Mgs/dl	0.6 – 1.2
Uric Acid	5.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	0.7	Mgs/dl	<1.0
Bilirubin Direct	0.1	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.6	Mgs/dl	0.2 to 1.0
SGOT/AST	31	U/L	0.0 to 40.0
SGPT/ALT	43	U/L	0.0 to 49.0
ALP	125	U/L	40.0 to 129.0
Gamma GT(GGT)	19	U/L	7.0 to 38.0
Protein	6.9	g/dl	6.0 to 8.0
Albumin	3.8	g/dl	3.5 to 5.0
Globulin	3.4	g/dl	1.8 to 3.5
A/G Ratio	2.1	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

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LABORATORY INVESTIGATION REPORT

Group 6-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	37	Mgs/dl	15 - 45
Creatinine	0.6	Mgs/dl	0.6 – 1.2
Uric Acid	5.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	0.7	Mgs/dl	<1.0
Bilirubin Direct	0.1	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.6	Mgs/dl	0.2 to 1.0
SGOT/AST	31	U/L	0.0 to 40.0
SGPT/ALT	43	U/L	0.0 to 49.0
ALP	120	U/L	40.0 to 129.0
Gamma GT(GGT)	19	U/L	7.0 to 38.0
Protein	6.9	g/dl	6.0 to 8.0
Albumin	3.8	g/dl	3.5 to 5.0
Globulin	3.4	g/dl	1.8 to 3.5
A/G Ratio	2.1	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

Dr. N. Madhivanan M.D.,(Bio) **Dr. V.P. Sarasu,** M.D., (Micro) **Dr. V. Sarada** M.D., (Path)

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LABORATORY INVESTIGATION REPORT

Group 7-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	37	Mgs/dl	15 - 45
Creatinine	0.6	Mgs/dl	0.6 – 1.2
Uric Acid	5.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	0.7	Mgs/dl	<1.0
Bilirubin Direct	0.1	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.6	Mgs/dl	0.2 to 1.0
SGOT/AST	31	U/L	0.0 to 40.0
SGPT/ALT	43	U/L	0.0 to 49.0
ALP	125	U/L	40.0 to 129.0
Gamma GT(GGT)	19	U/L	7.0 to 38.0
Protein	6.9	g/dl	6.0 to 8.0
Albumin	3.8	g/dl	3.5 to 5.0
Globulin	3.4	g/dl	1.8 to 3.5
A/G Ratio	2.1	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

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LABORATORY INVESTIGATION REPORT

Group 8-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	37	Mgs/dl	15 - 45
Creatinine	0.6	Mgs/dl	0.6 – 1.2
Uric Acid	5.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	0.7	Mgs/dl	<1.0
Bilirubin Direct	0.1	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.6	Mgs/dl	0.2 to 1.0
SGOT/AST	31	U/L	0.0 to 40.0
SGPT/ALT	43	U/L	0.0 to 49.0
ALP	120	U/L	40.0 to 129.0
Gamma GT(GGT)	19	U/L	7.0 to 38.0
Protein	6.9	g/dl	6.0 to 8.0
Albumin	3.8	g/dl	3.5 to 5.0
Globulin	3.4	g/dl	1.8 to 3.5
A/G Ratio	2.1	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

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LABORATORY INVESTIGATION REPORT

Group 9-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	37	Mgs/dl	15 - 45
Creatinine	0.6	Mgs/dl	0.6 – 1.2
Uric Acid	5.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	0.1	Mgs/dl	<1.0
Bilirubin Direct	0.5	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.6	Mgs/dl	0.2 to 1.0
SGOT/AST	31	U/L	0.0 to 40.0
SGPT/ALT	43	U/L	0.0 to 49.0
ALP	125	U/L	40.0 to 129.0
Gamma GT(GGT)	19	U/L	7.0 to 38.0
Protein	6.9	g/dl	6.0 to 8.0
Albumin	3.8	g/dl	3.5 to 5.0
Globulin	3.4	g/dl	1.8 to 3.5
A/G Ratio	2.1	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

Dr. N. Madhivanan M.D., (Bio) Dr. V.P. Sarasu, M.D., (Micro) Dr. V. Sarada M.D., (Path)

Get Well Soon

FRIENDS DIAGNOSTIC CENTRE
A Group of Ashka Friends Diagnostic (P) Ltd.,
Multi Speciality Referral Lab

Plot No.20, Ist Floor, Sundaram Building,
Opp. Baba Towers, Sastri Road, Trichy.
Ph : +91 431 4220877, Mobile : 99945 32390

LABORATORY INVESTIGATION REPORT

Group 10-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	37	Mgs/dl	15 - 45
Creatinine	0.6	Mgs/dl	0.6 – 1.2
Uric Acid	5.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	1.1	Mgs/dl	<1.0
Bilirubin Direct	0.5	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.6	Mgs/dl	0.2 to 1.0
SGOT/AST	31	U/L	0.0 to 40.0
SGPT/ALT	43	U/L	0.0 to 49.0
ALP	125	U/L	40.0 to 129.0
Gamma GT(GGT)	19	U/L	7.0 to 38.0
Protein	6.9	g/dl	6.0 to 8.0
Albumin	3.8	g/dl	3.5 to 5.0
Globulin	3.4	g/dl	1.8 to 3.5
A/G Ratio	2.1	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

FRIENDS DIAGNOSTIC CENTRE
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Ph : +91 431 4220877, Mobile : 99945 32390

LABORATORY INVESTIGATION REPORT

Group 11-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

Blood Urea (BUN)	37	Mgs/dl	15 - 45
Creatinine	0.6	Mgs/dl	0.6 – 1.2
Uric Acid	5.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	1.1	Mgs/dl	<1.0
Bilirubin Direct	0.5	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.6	Mgs/dl	0.2 to 1.0
SGOT/AST	31	U/L	0.0 to 40.0
SGPT/ALT	43	U/L	0.0 to 49.0
ALP	125	U/L	40.0 to 129.0
Gamma GT(GGT)	19	U/L	7.0 to 38.0
Protein	6.9	g/dl	6.0 to 8.0
Albumin	3.8	g/dl	3.5 to 5.0
Globulin	3.4	g/dl	1.8 to 3.5
A/G Ratio	2.1	No	1.2 to 2.5

Please Correlate with Clinical Conditions End of the Report

FRIENDS DIAGNOSTIC CENTRE
A Group of Ashka Friends Diagnostic (P) Ltd.,
Multi Speciality Referral Lab

Plot No.20, Ist Floor, Sundaram Building,
Opp. Baba Towers, Sastri Road, Trichy.
Ph : +91 431 4220877, Mobile : 99945 32390

LABORATORY INVESTIGATION REPORT

Group 12-1

LABORATORY INVESTIGATION REPORT

Final Test Report

TEST NAME	RESULT	UNITS	NORMAL RANGES

BIO – CHEMISTRY:

RFT

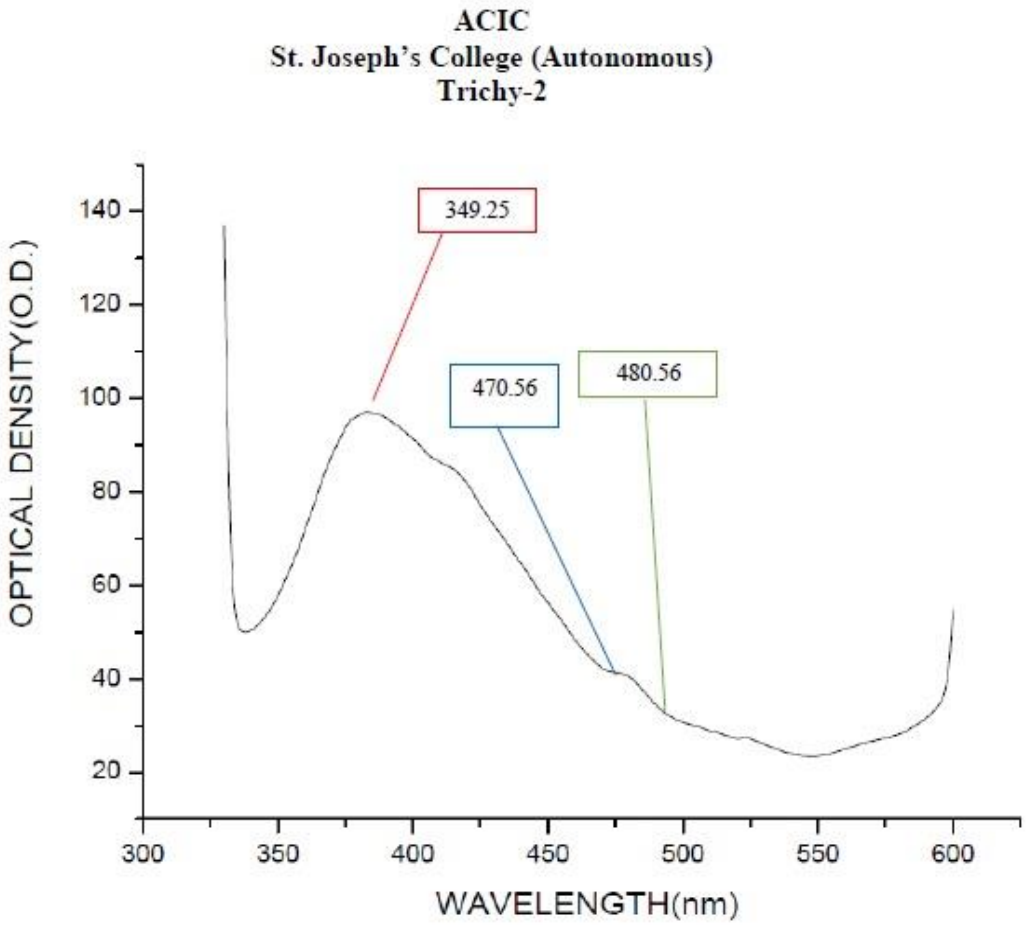
Blood Urea (BUN)	37	Mgs/dl	15 - 45
Creatinine	0.6	Mgs/dl	0.6 – 1.2
Uric Acid	5.1	Mgs/dl	2.6 – 7.2

LFT

Bilirubin Total	0.7	Mgs/dl	<1.0
Bilirubin Direct	0.1	Mgs/dl	0.0 to 0.3
Bilirubin Indirect	0.6	Mgs/dl	0.2 to 1.0
SGOT/AST	31	U/L	0.0 to 40.0
SGPT/ALT	43	U/L	0.0 to 49.0
ALP	125	U/L	40.0 to 129.0
Gamma GT(GGT)	19	U/L	7.0 to 38.0
Protein	6.9	g/dl	6.0 to 8.0
Albumin	3.8	g/dl	3.5 to 5.0
Globulin	3.4	g/dl	1.8 to 3.5
A/G Ratio	2.1	No	1.2 to 2.5

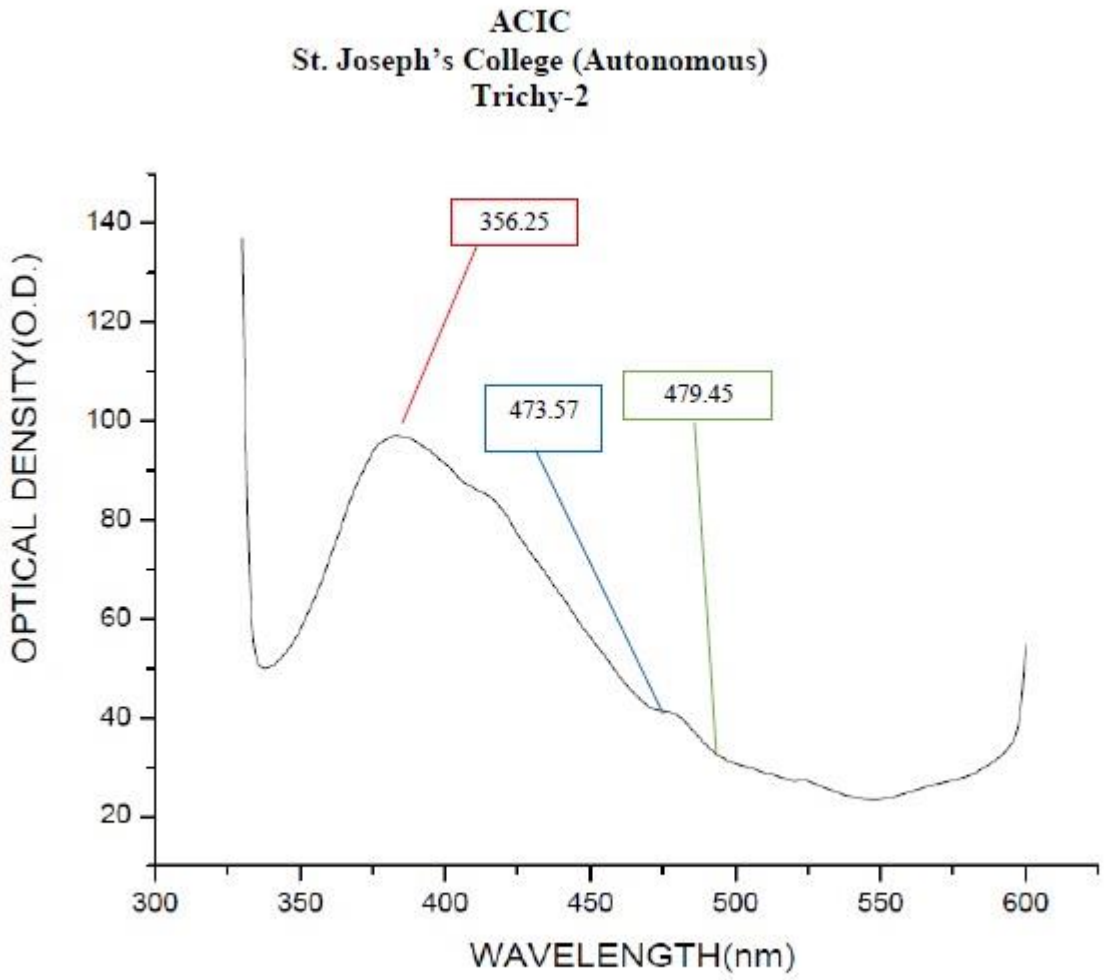
Please Correlate with Clinical Conditions End of the Report

C) FLUORESENCE SPECTROFLUORIMETRY

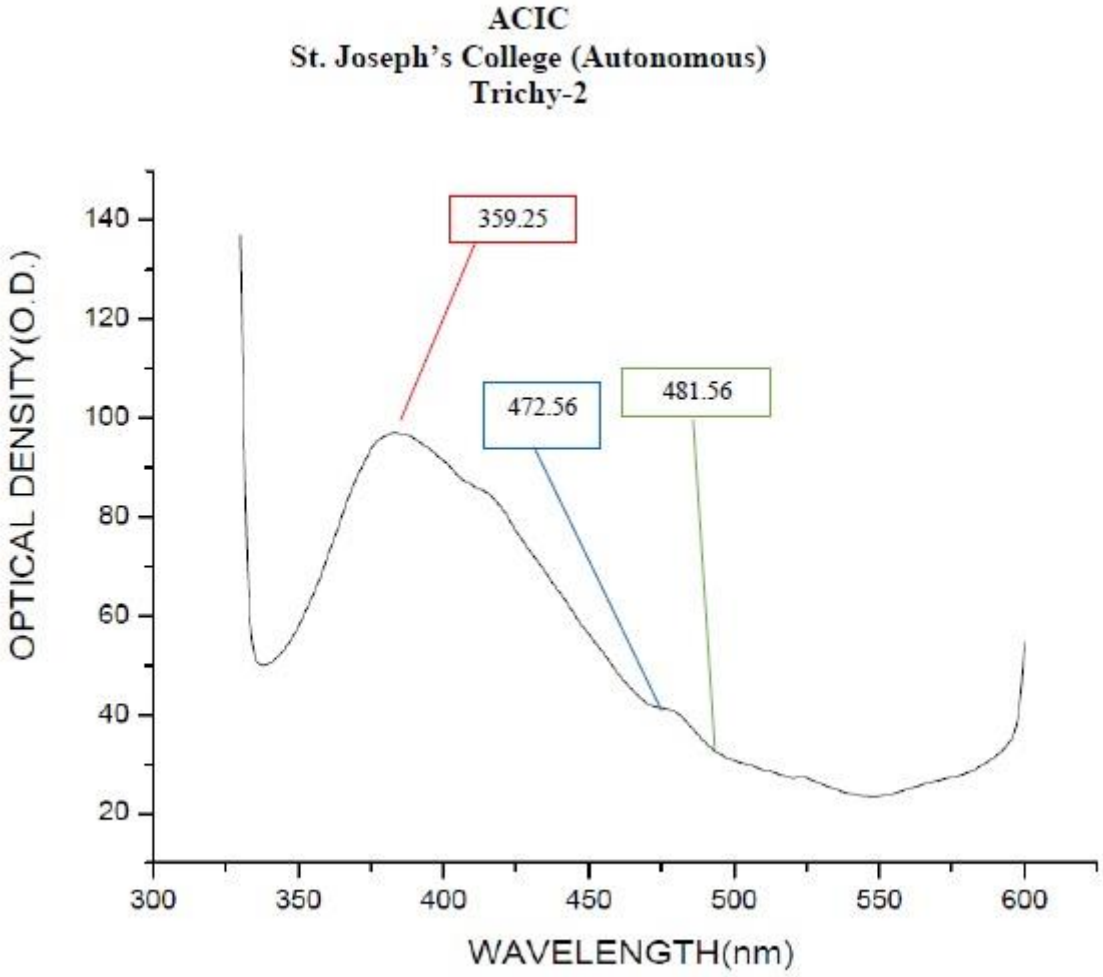


GROUP 1: 1

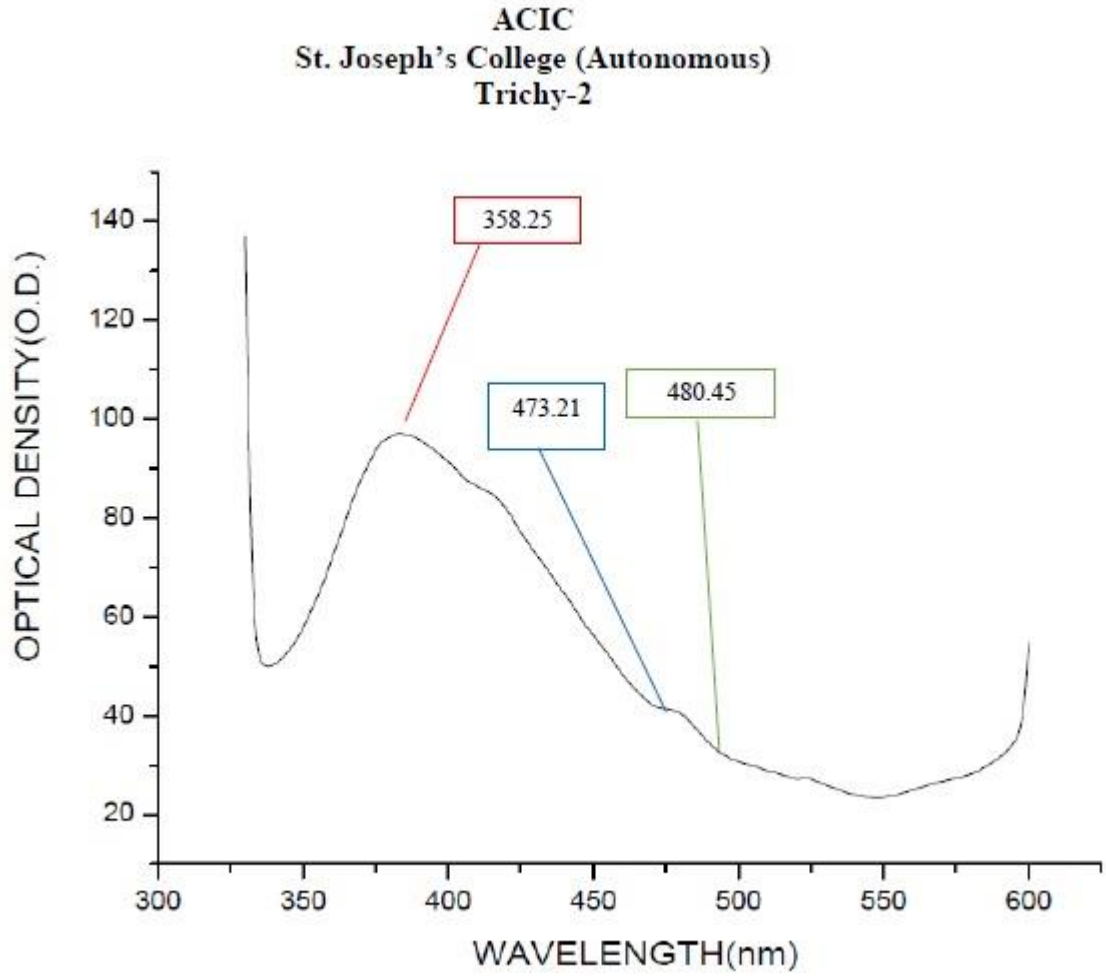
GROUP 1: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline



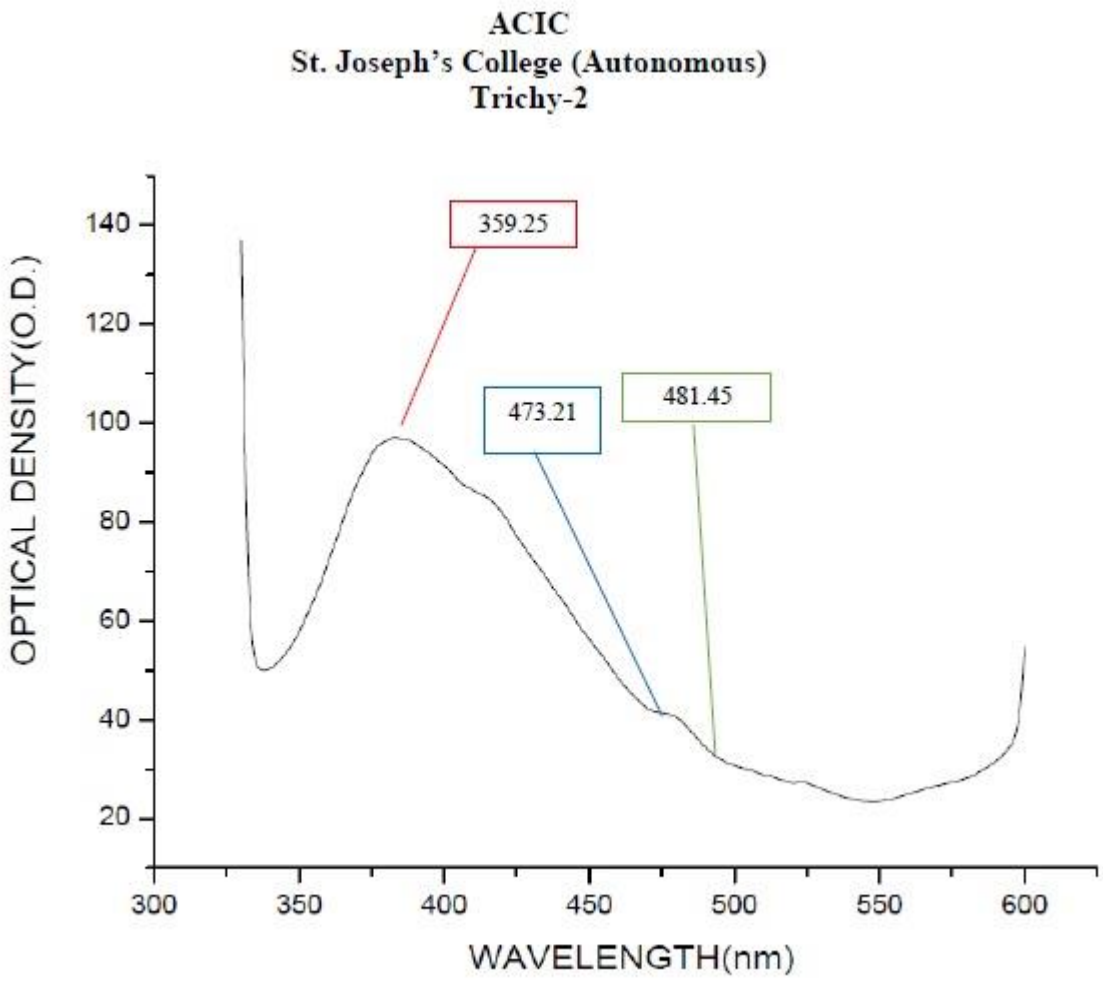
GROUP 2: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline



GROUP 3: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline

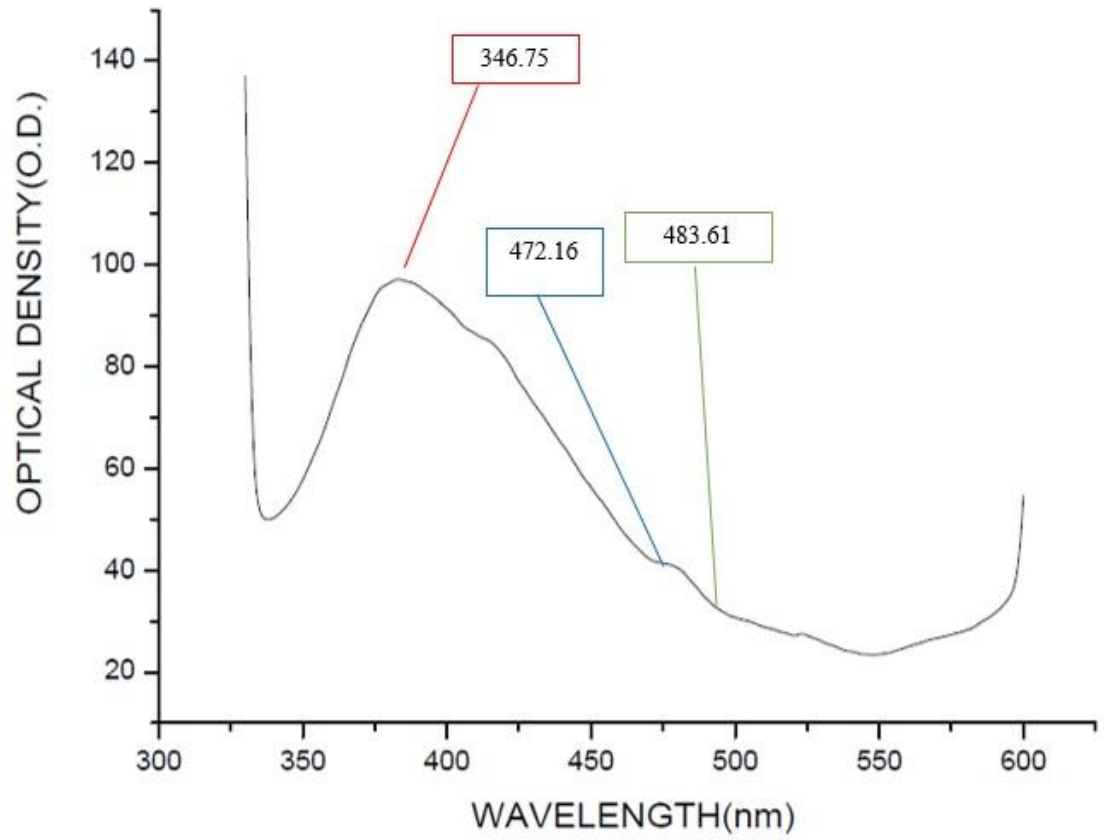


GROUP 4: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline



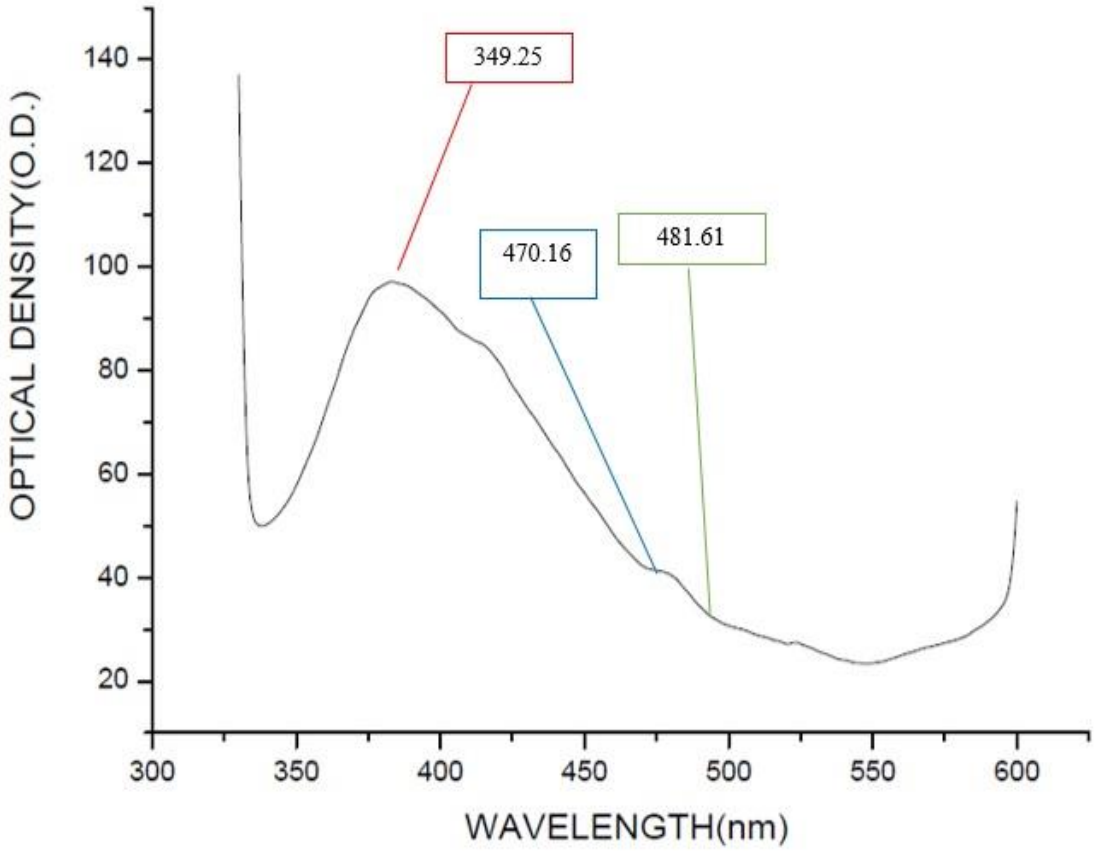
GROUP 5: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline

ACIC
St. Joseph's College (Autonomous)
Trichy-2

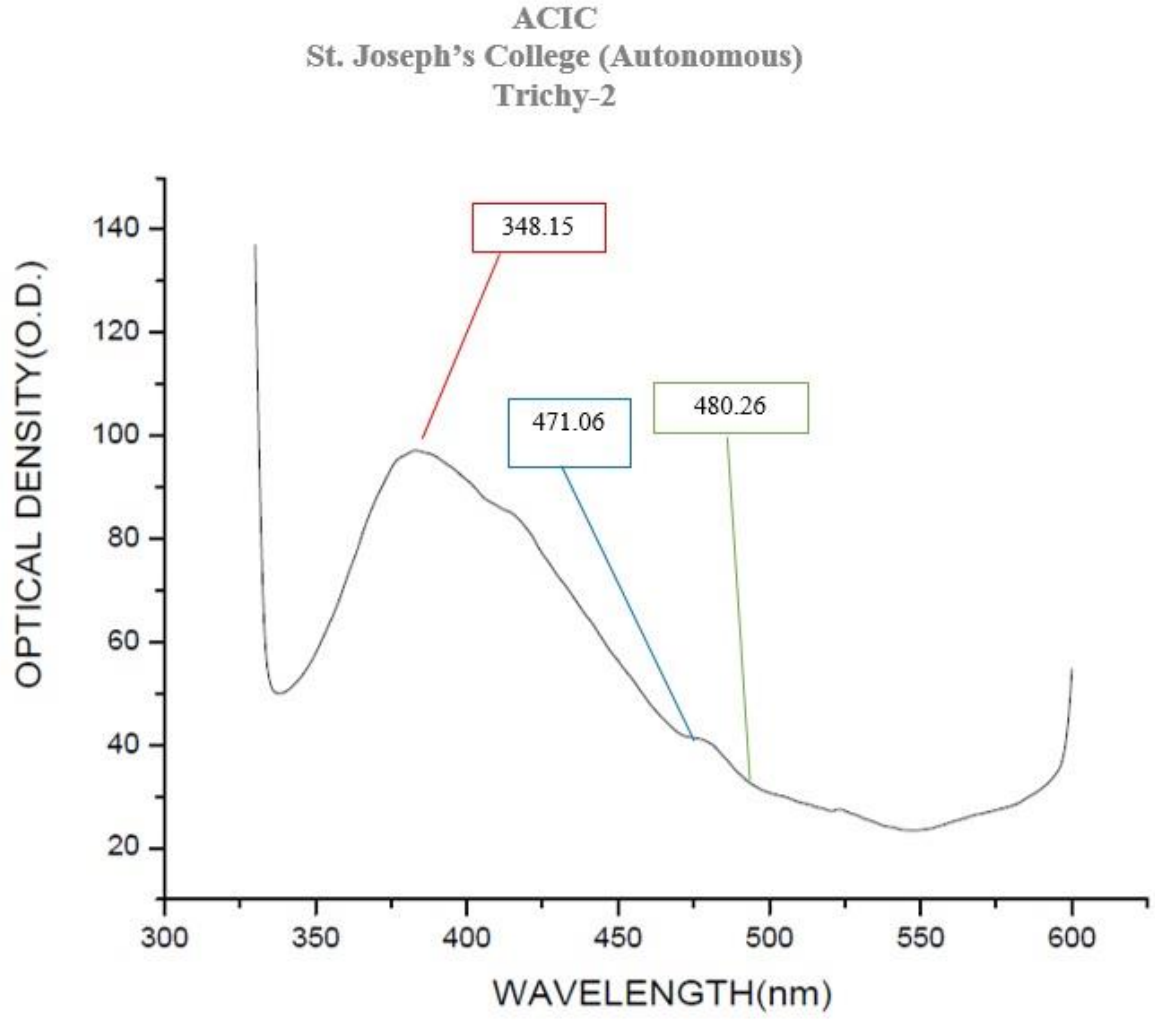


GROUP 6: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline

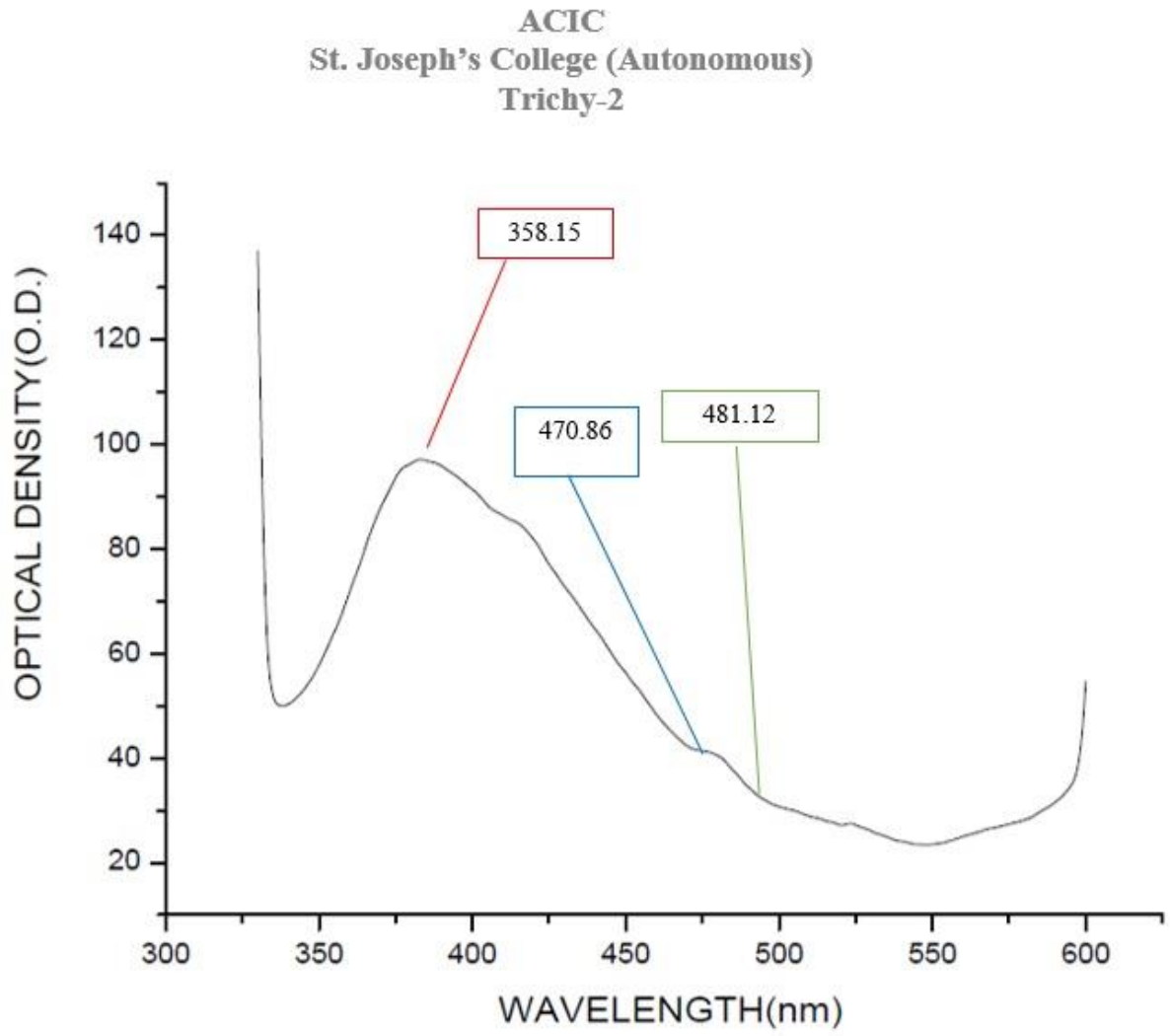
ACIC
St. Joseph's College (Autonomous)
Trichy-2



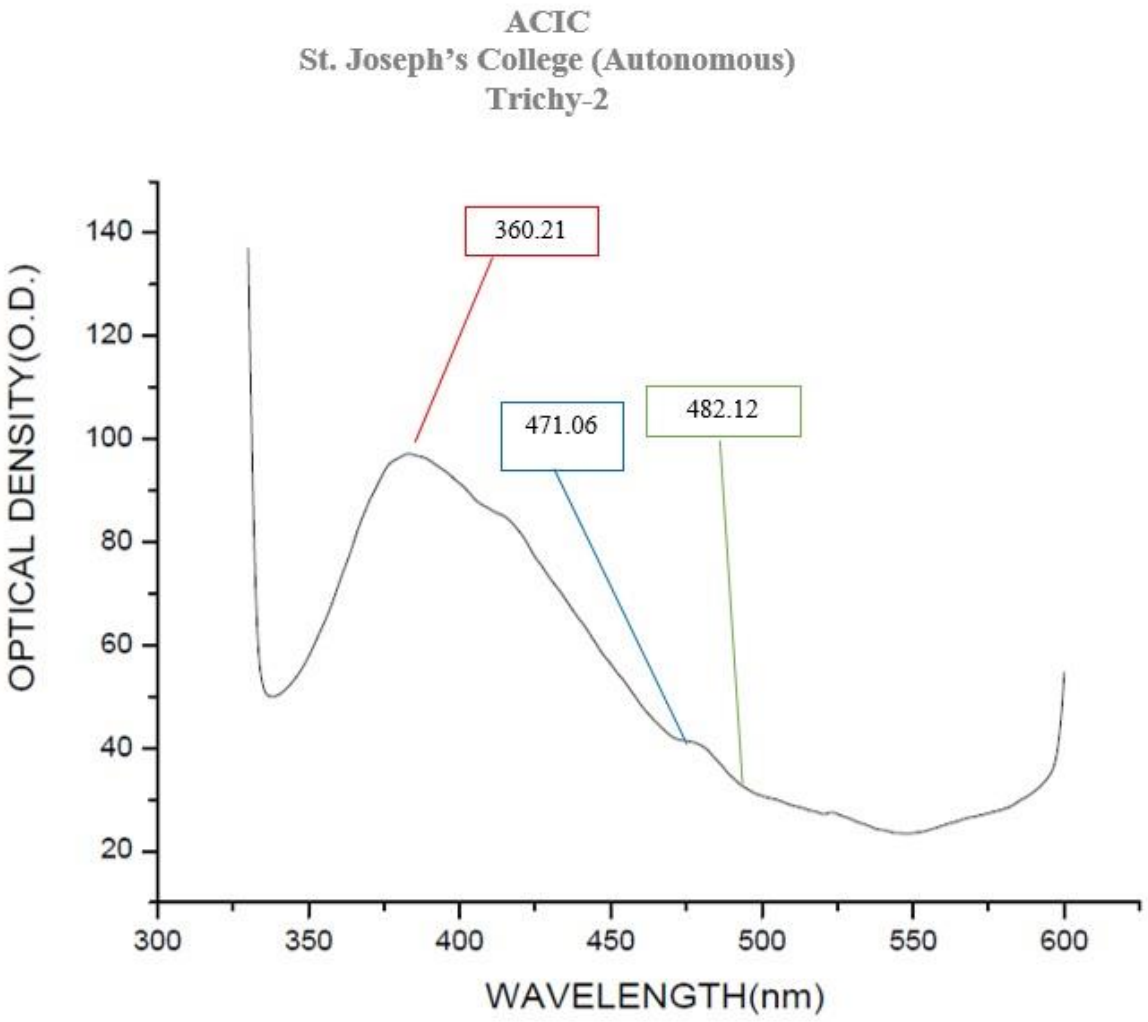
GROUP 7: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline



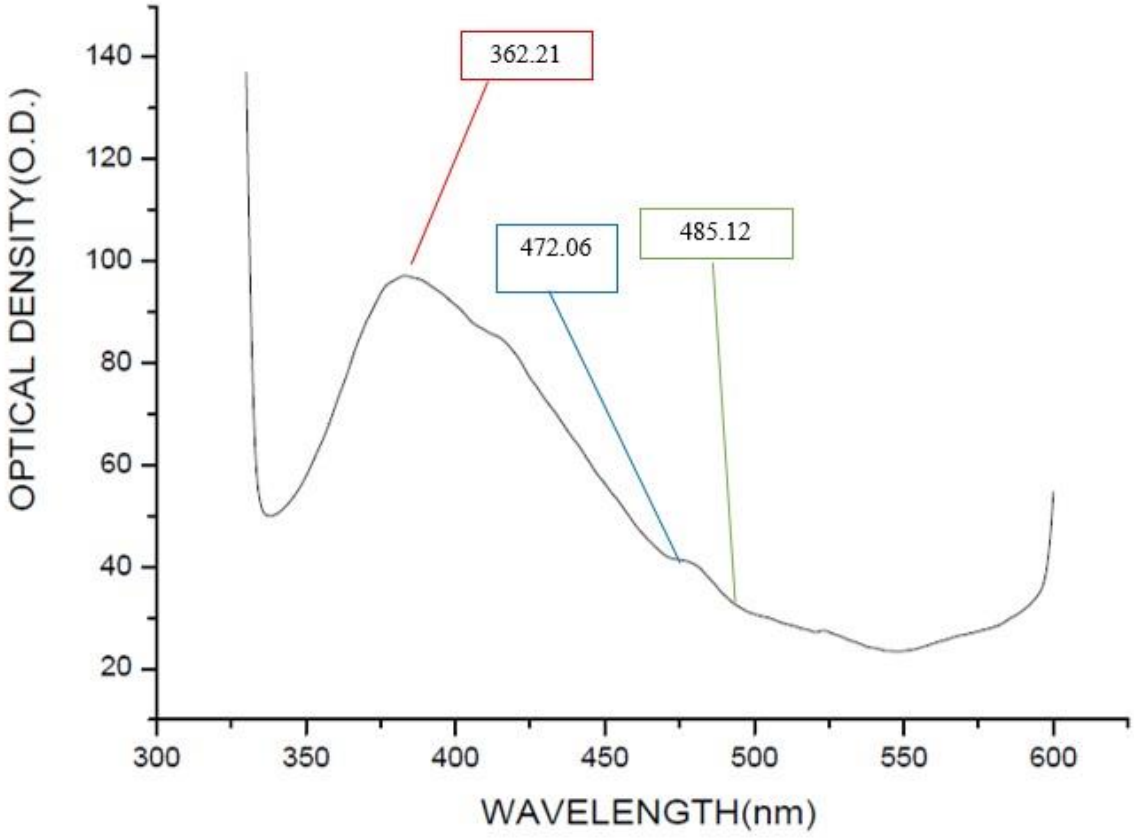
GROUP 8: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline



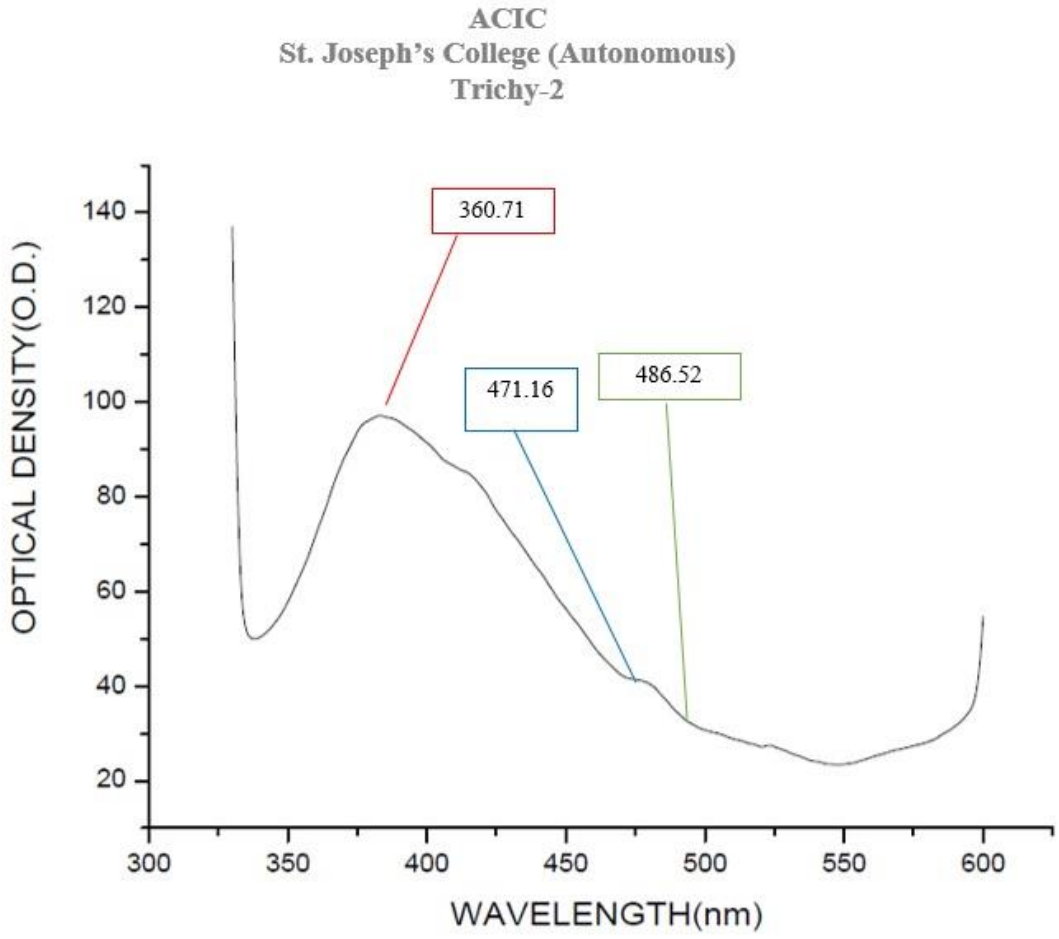
GROUP 9: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline



GROUP 10: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline

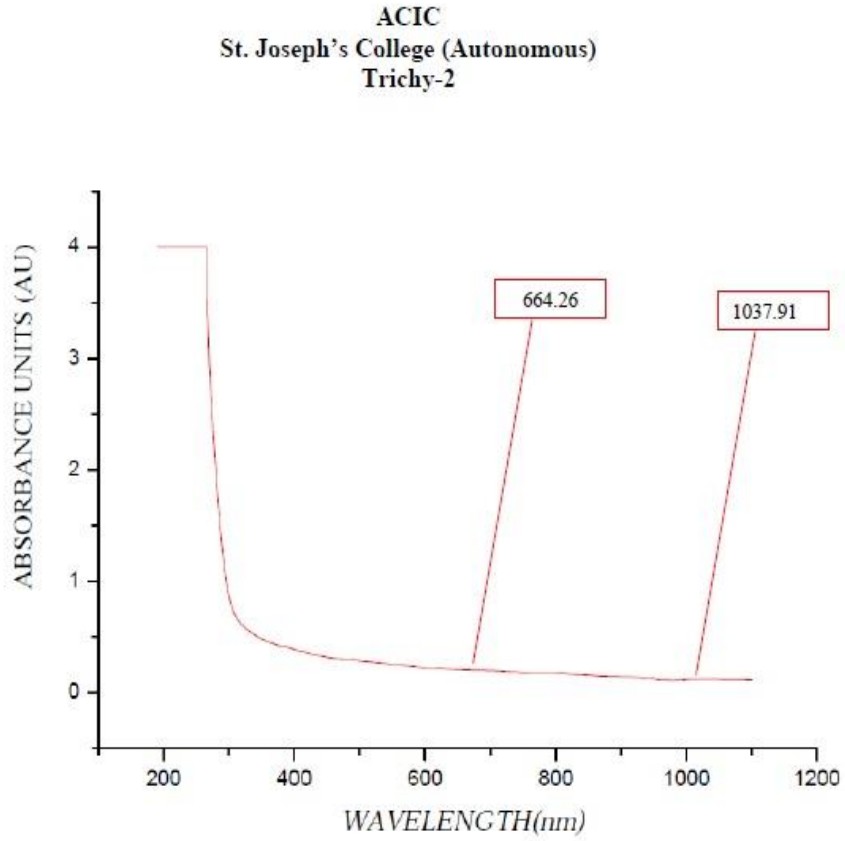


GROUP 11: Graph showing the Optical Densities of neurotransmitter values of

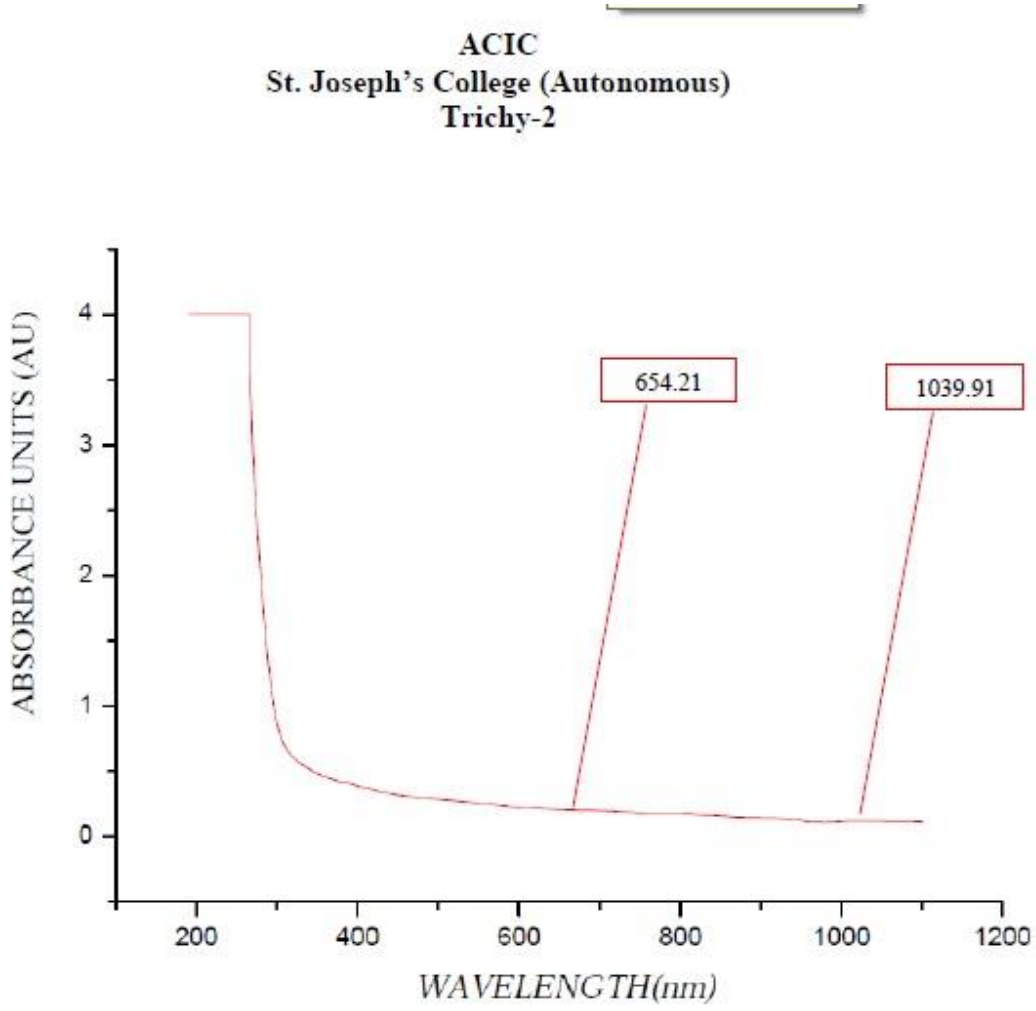


GROUP 12: Graph showing the Optical Densities of neurotransmitter values of Dopamine, Serotonin and Noradrenaline

D) UV VISIBLE SPECTROPHOTOMETRY

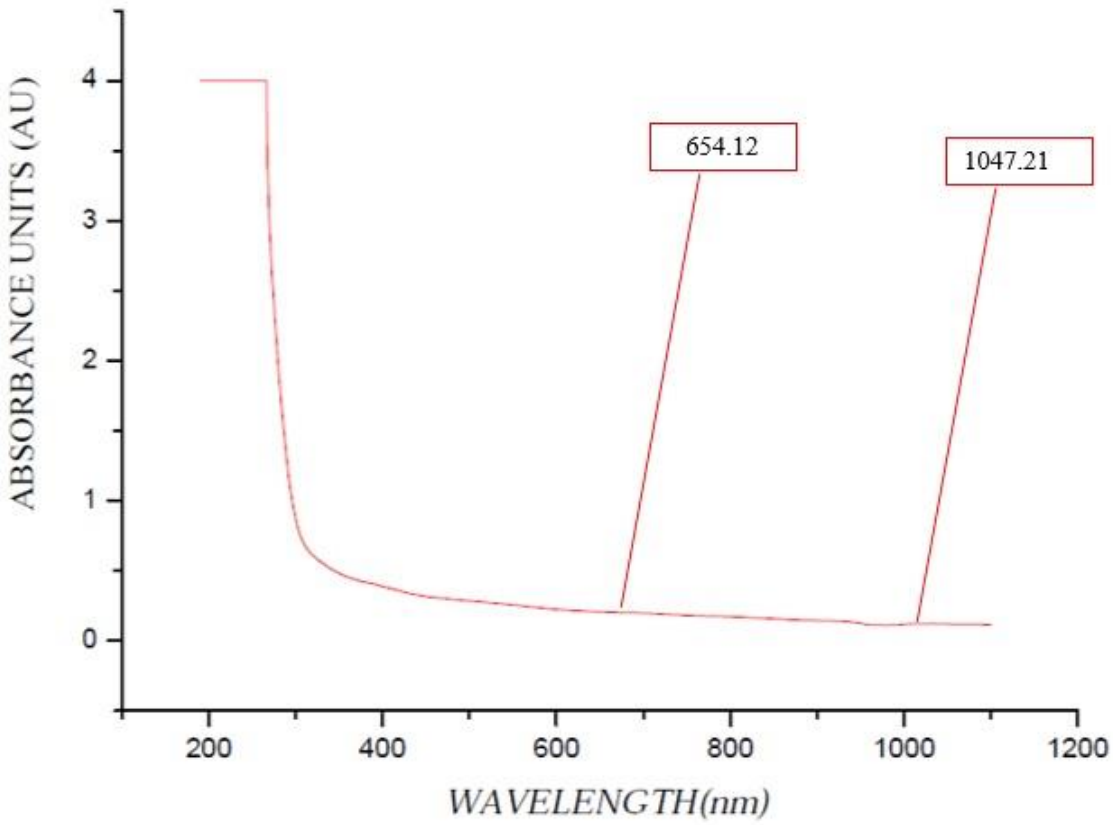


GROUP 1: Graph showing 1 minute and 2 minutes absorbance units for Acetylcholinesterase levels



GROUP 2: Graph showing 1 minute and 2 minutes absorbance units for Acetylcholinesterase levels

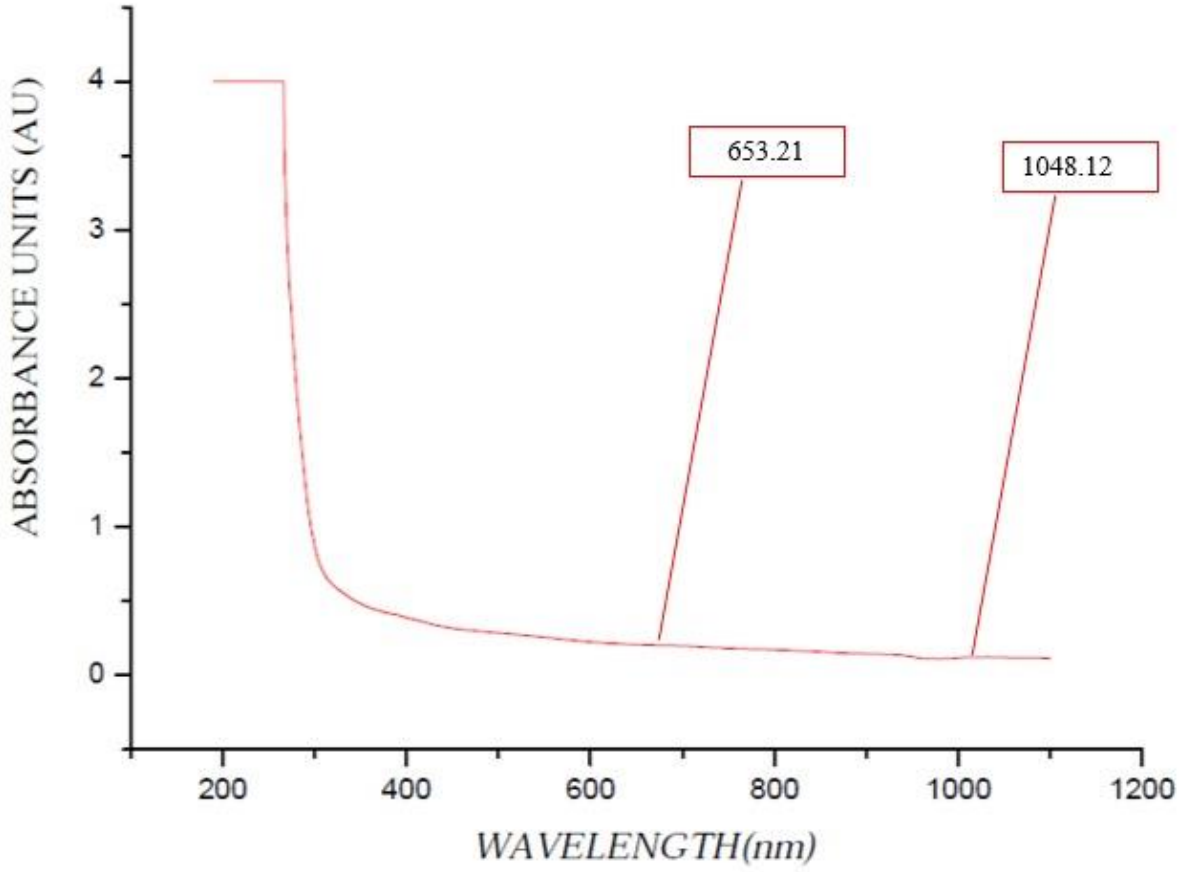
ACIC
St. Joseph's College (Autonomous)
Trichy-2



GROUP 3: Graph showing 1 minute and 2 minutes absorbance units for

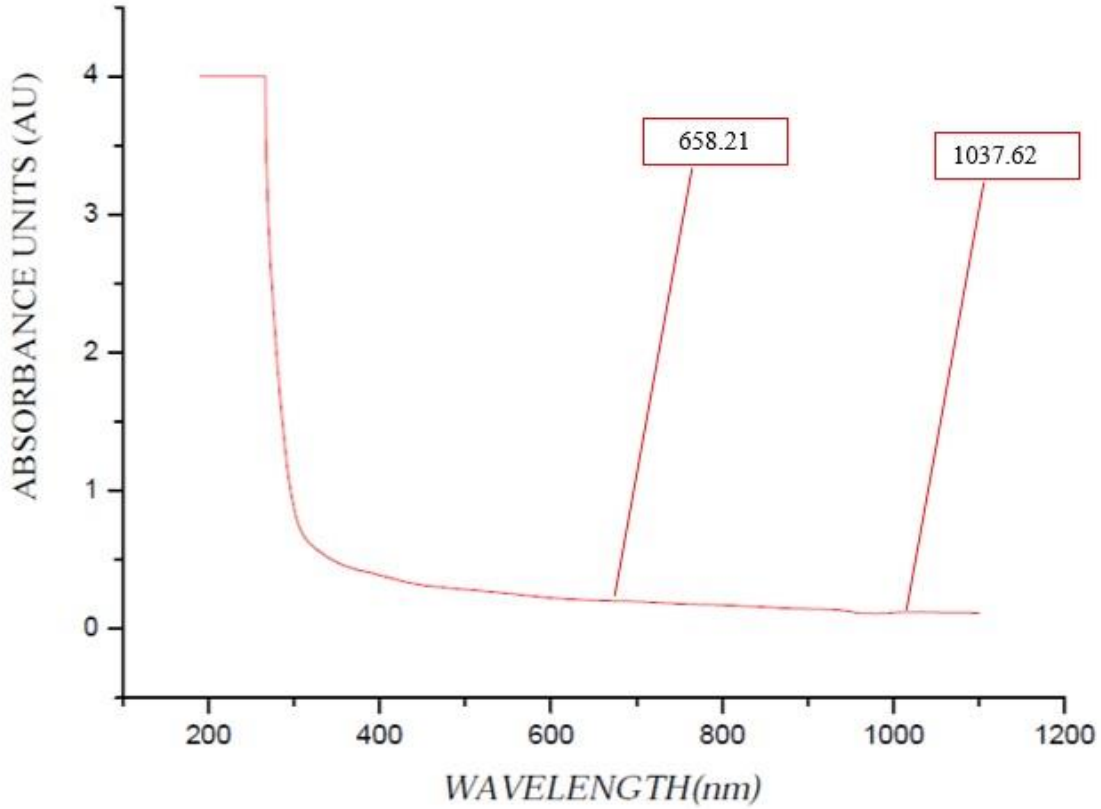
Acetylcholinesterase levels

ACIC
St. Joseph's College (Autonomous)
Trichy-2



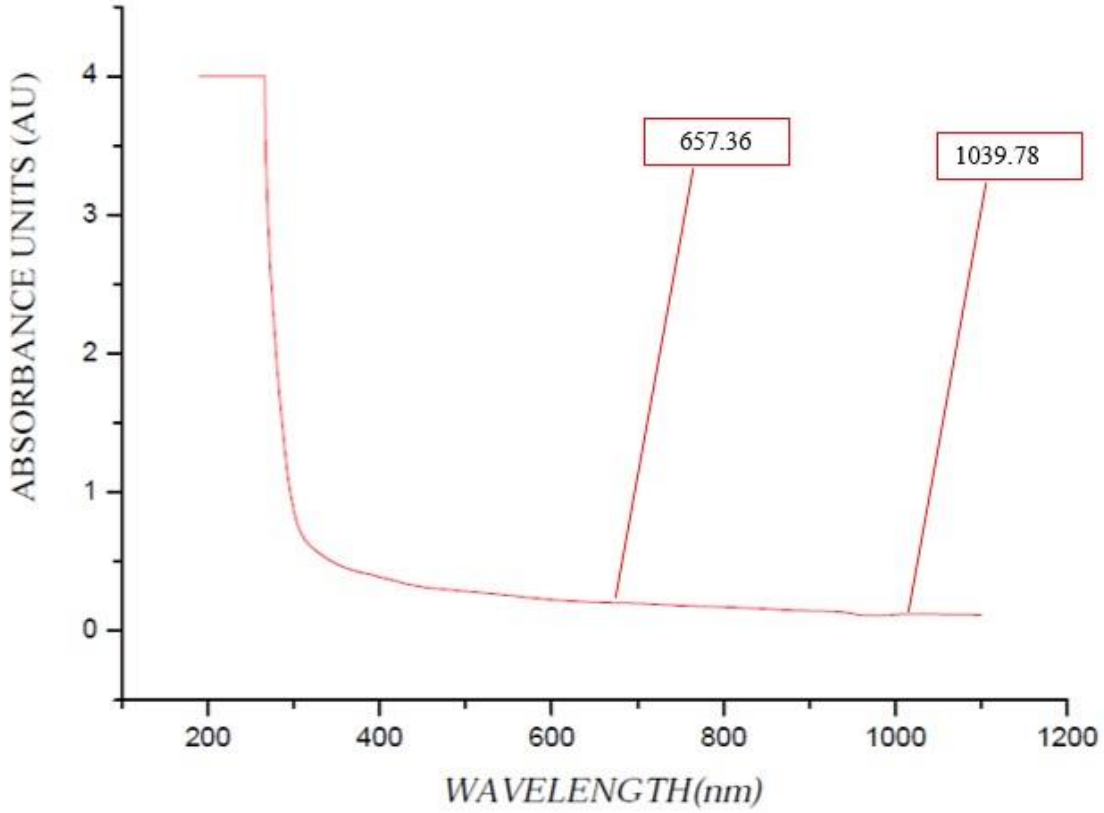
GROUP 4: Graph showing 1 minute and 2 minutes absorbance units for Acetylcholinesterase levels

ACIC
St. Joseph's College (Autonomous)
Trichy-2



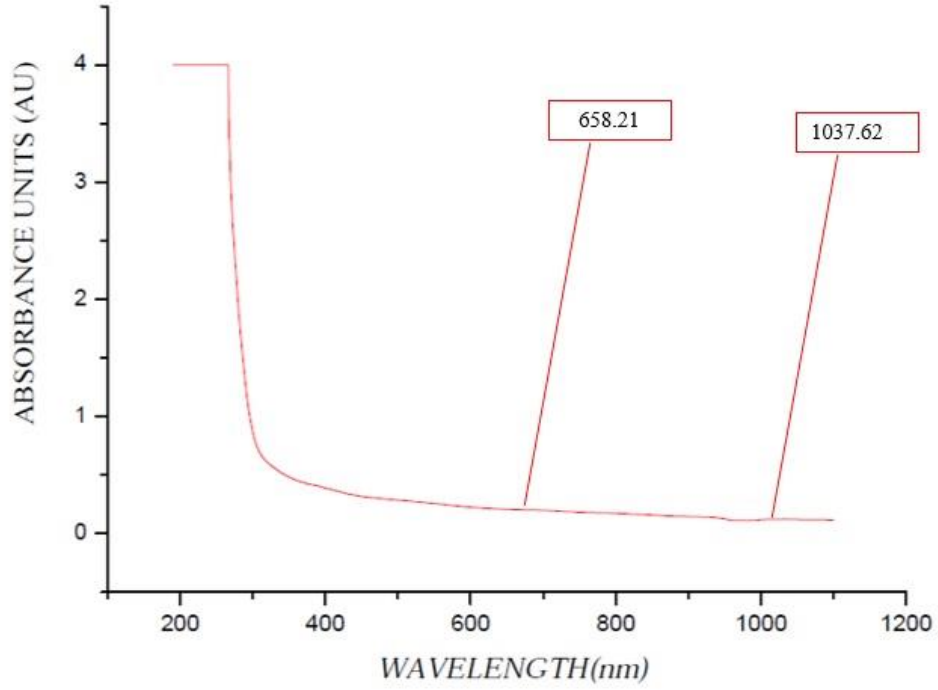
GROUP 5: Graph showing 1 minute and 2 minutes absorbance units for Acetylcholinesterase levels

ACIC
St. Joseph's College (Autonomous)
Trichy-2



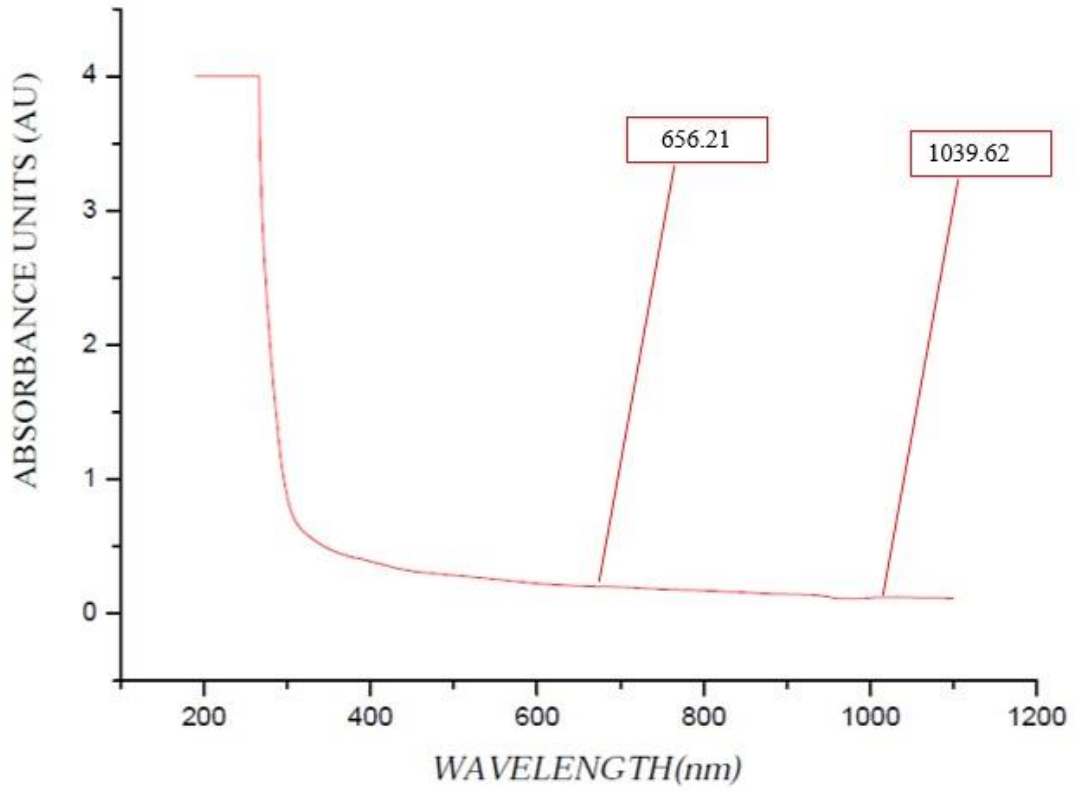
GROUP 6: Graph showing 1 minute and 2 minutes absorbance units for

ACIC
St. Joseph's College (Autonomous)
Trichy-2



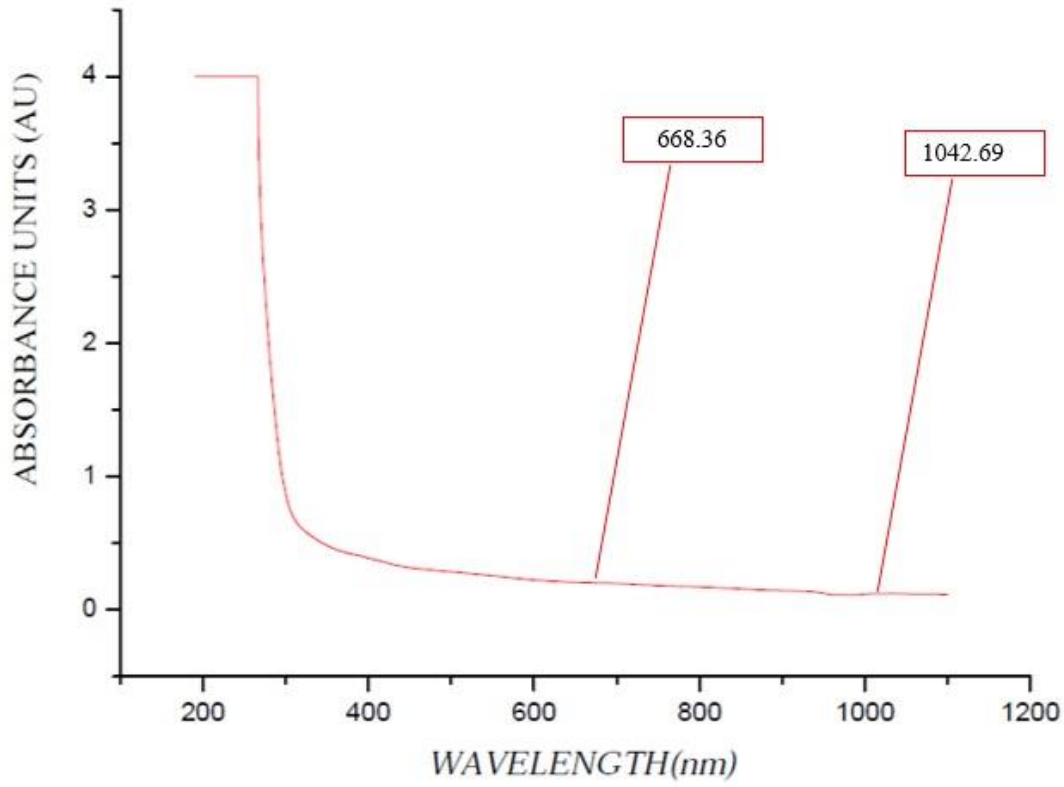
GROUP 7: Graph showing 1 minute and 2 minutes absorbance units for Acetylcholinesterase levels

ACIC
St. Joseph's College (Autonomous)
Trichy-2



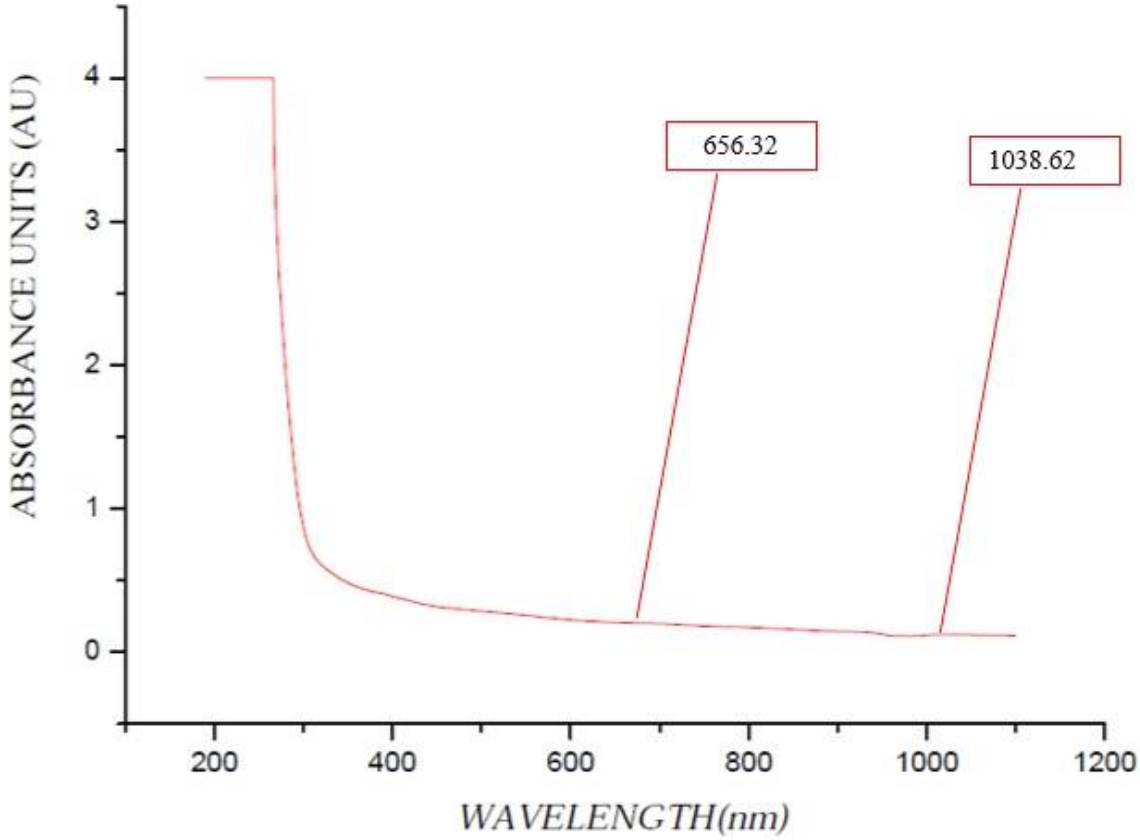
GROUP 8: Graph showing 1 minute and 2 minutes absorbance units for Acetylcholinesterase levels

ACIC
St. Joseph's College (Autonomous)
Trichy-2



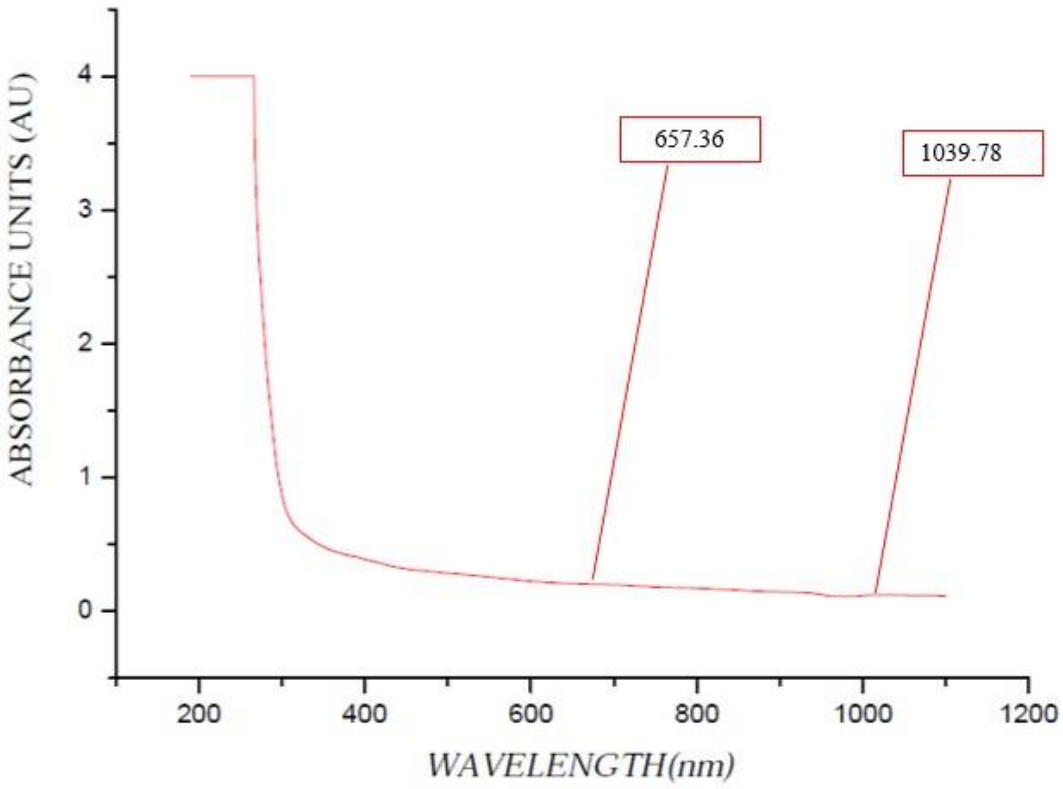
GROUP 9: Graph showing 1 minute and 2 minutes absorbance units for Acetylcholinesterase levels

ACIC
St. Joseph's College (Autonomous)
Trichy-2



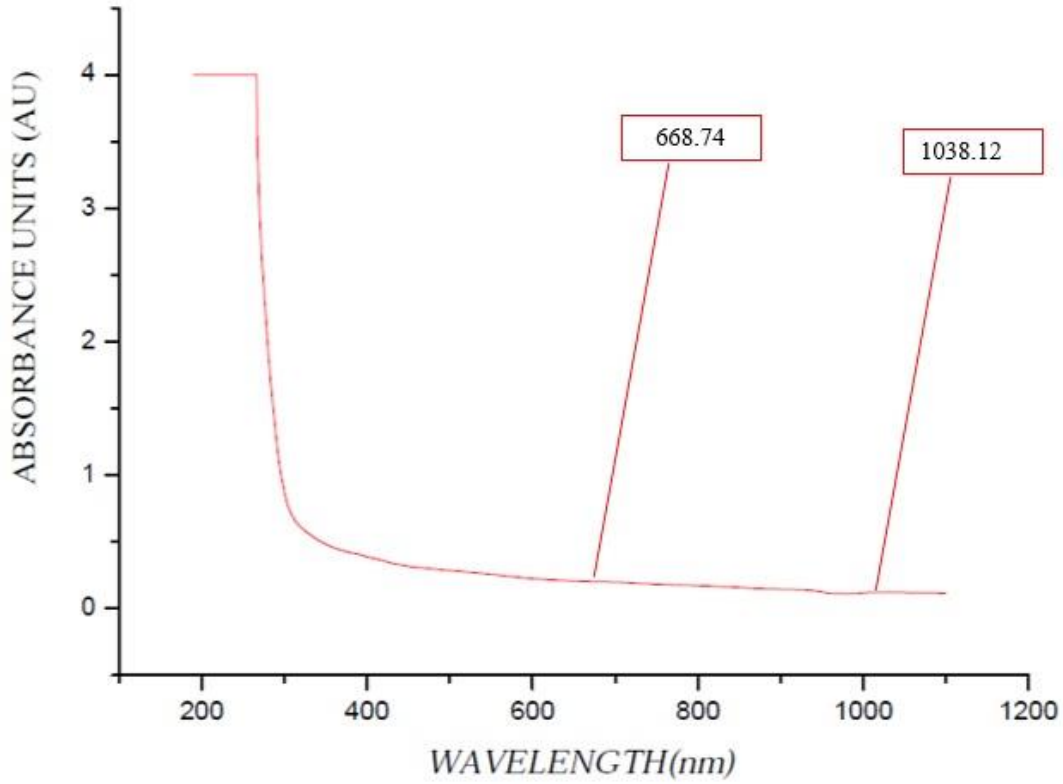
GROUP 10: Graph showing 1 minute and 2 minutes absorbance units for Acetylcholinesterase levels

ACIC
St. Joseph's College (Autonomous)
Trichy-2



GROUP 11: Graph showing 1 minute and 2 minutes absorbance units for Acetylcholinesterase levels

ACIC
St. Joseph's College (Autonomous)
Trichy-2



GROUP 12: Graph showing 1 minute and 2 minutes absorbance units for Acetylcholinesterase levels

ANNEXURE-II

BIBILIOGRAPHY:

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